Domestic Wastewater Treatment with Integrated Constructed Wetland – Microbial Fuel Cell Emphasizing Organics and Coliform Remediation with Bioelectricity Generation

Ph.D. Thesis

Submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Submitted by

Anjishnu Biswas

(186104005)

Under the supervision of

Professor Saswati Chakraborty

Department of Civil Engineering

Indian Institute of Technology Guwahati

Guwahati - 781039, Assam, India

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Dedicated to the memories of my father, the sacrifices of my mother, and the endeavours of all the research scholars who strive each day to contribute to the field of SCIENCE…

Statement

I hereby declare that the content embodied in this thesis entitled **Domestic Wastewater Treatment with Integrated Constructed Wetland – Microbial Fuel Cell Emphasizing Organics and Coliform Remediation with Bioelectricity Generation** is the documentation of the outcomes of the investigations performed by me in the Department of Civil Engineering, Indian Institute of Technology Guwahati, Guwahati, Assam, India under the supervision of **Prof. Saswati Chakraborty**. Adhered to the general practice of reporting scientific observations, due acknowledgments have been mentioned wherever the work described is based on the findings of other investigators and researchers.

A. Biswas

Dated: 31st of January 2025

Place: Guwahati

Anjishnu Biswas

Roll No. 186104005

Department of Civil Engineering

Indian Institute of Technology Guwahati

Guwahati, Assam, India, 781039

Indian Institute of Technology Guwahati

Department of Civil Engineering

Certificate

It is certified that the research work documented in this thesis entitled **Domestic Wastewater Treatment with Integrated Constructed Wetland – Microbial Fuel Cell Emphasizing Organics and Coliform Remediation with Bioelectricity Generation**, carried out by **Mr. Anjishnu Biswas** (Roll No. 186104005) for the award of the degree of Doctor of Philosophy is an authentic record of the results obtained from the experimental works carried out under my supervision in the Department of Civil Engineering, Indian Institute of Technology Guwahati, Assam, India. I certify that he has completed all the requirements as per the rules of this Institute and this work has not been submitted elsewhere for consideration for any academic degree.

Dated: 31st of January 2025

Place: Guwahati

Dr. Saswati Chakraborty

Professor

Department of Civil Engineering

Indian Institute of Technology Guwahati

Guwahati, Assam, India, 781039

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A. Biswas

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Abstract

India as a nation is facing a serious concern due to the lack of domestic wastewater treatment capacity in the urban and rural sectors. Rapid urbanization followed by industrialization has increased the demand for fresh water and subsequent production of wastewater. The centralized wastewater treatment capacity of India marks up to 30% in the urban sectors and the situation in the rural and suburban regions is worse. Traditional wastewater treatment technologies are primarily designed for the reduction of organic loading in domestic wastewater and are less focused on reducing fecal contamination. This is a major hurdle for the reuse of treated wastewater. Decentralized wastewater treatment schemes could be a solution having low energy requirements with little operation and maintenance needs. The integrated constructed wetland microbial fuel cell (CW-MFC) technology serves the purpose of eco-friendly wastewater treatment with the potential of reducing the areal footprint of constructed wetlands along with high-rate organic removal, low maintenance, and the scope of bioenergy harvesting. Therefore this research was conducted for the treatment of domestic wastewater using an integrated CW-MFC reactor for organics and coliform removal with bioelectricity generation and for understanding the inherent bioelectrochemical mechanisms. The probable economic benefits of the CW-MFC system were also assessed using a cost-benefit analysis study.

The removal of organics and fecal coliforms from domestic wastewater was evaluated using three identical reactors. One vertical planted constructed wetland (R1), one planted CW-MFC (R2), and one unplanted CW-MFC (R3). In terms of COD removal, R2 achieved 93.13±4.83% efficiency, and R1 and R3 achieved 87.21±4.56% and 86.31±4.37% efficiencies, respectively. BOD_5 removal efficiency in R1, R2, and R3 reactors was found to be similar from 91.79±3.58% to 92.7±3.3%. The fecal coliform removal in R2 was much higher (log 3.51) compared to R1 (log 2.94). Coliform and organic removal deteriorated during the winter, though electricity generation remained unaffected. The maximum open-circuit voltage in R2 was 886 mV and in R3 was 487 mV during the complete study. *Typha angustifolia* played a positive role in enhancing the electrical performance of R2 compared to unplanted R3 by increasing the redox potential near the cathode. The presence of dissolved oxygen in the range

of 1.97–2.1 mg/L was also detected near the rhizosphere of the planted reactors which worked as a terminal electron acceptor and helped in completing the cathode reaction.

Spatial analysis of treated effluents showed a considerable amount of organics and fecal coliform removal at the vicinity of the anode in R2. Assessment of the microbial population inside all the reactors revealed that electroactive bacteria (EAB) (*Firmicutes, Bacteroidetes, and Actinobacteria*) were more abundant in R2 compared to R1 and R3. During the activity study, biomass obtained from R2 showed a maximum substrate utilization rate of 1.27 mg COD $mgVSS^{-1}$ d^{-1}. Kinetic batch studies were carried out for fecal coliform removal in all the reactors, and the maximum first-order fecal coliform removal rate was obtained at the anode of R2 during closed circuit mode. Simultaneous bioelectricity monitoring inferred that voltage generation can be correlated to faster fecal coliform inactivation, which was probably due to EABs outcompeting other exogenous microbes in a preferable anaerobic environment with the presence of an anode.

Periodic reduction of hydraulic retention time (HRT) resulted in deteriorated organics and fecal coliform removal by the reactors. R2 showed higher pollutant removal and voltage generation throughout as compared to R1 and R3. R2 exerted 93%, 87%, and 57% COD removal during the HRT of 36 h (Phase 1), 24 h (Phase 2), and 18 h (Phase 3); with maximum voltage generated as 865 mV, 695 mV, and 429 mV respectively. Multiple batch studies revealed the main role of reactor media and plant roots was to support the attached microbial growth for biodegradation of the organics. Radial oxygen loss did not affect the anaerobic environment at the anode after 800 days of reactor operation.

A comparative cost-benefit analysis of three reactors: R1, R2, and R3 was conducted with 36 h and 24 h HRT, and the respective cost-benefit ratio was determined. Results revealed that R2 was the most sustainable option with maximum economic benefits during Phase 1 and Phase 2 without effluent disinfection. With effluent disinfection in place, R1 emerged with the best cost-benefit ratio due to less investment cost and low revenue from electricity production in R2 and R3. Sensitivity analysis with net investment cost, power density, electrode area, effective reactor volume, and HRT; showed power density was the most influential parameter affecting the cost-benefit ratio. Revenue from wastewater treatment was found essential for economic sustainability which shows the importance of producing high-quality effluent and greater power density to make CW-MFC technology economically attractive.

Multi-anode CW-MFC reactors (both planted and unplanted and equipped with three anodes: A1, A2, and A3) were employed to compare the organics and fecal coliform removal performance and bioelectricity enhancement with that of single-anode CW-MFCs (planted and unplanted). The distance of anodes A1, A2, and A3 from the cathode was 20 cm, 15 cm, and 10 cm respectively. The multi-anode reactors were named R'1 (planted) and R'2 (unplanted), and the single-anode reactors were named R1 (planted) and R'1 (unplanted). In the case of multi-anode reactors the planted R'1 rendered better COD removal, fecal coliform inactivation, and operating voltage as compared to unplanted R'2. Compared to R1, R'1 showed 1.84% and 1.40% higher COD removal during closed-circuit and open-circuit operation. R'2 achieved 3.9% and 0.6% higher COD removal as compared to R2 during closed-circuit and open-circuit operation. The operating voltage and the power density were enhanced significantly in the multi-anode reactors as compared to single-anode reactors. For R'1 and R'2, different anodes rendered different operating voltages depending on the COD availability and the respective distance from the cathode. Anode A2 which was placed at an intermediate position in between anodes A1 and A3, showed the maximum operating voltage (791 mV) and power density (7.47 mW/m^2). The fecal coliform removal efficiencies of all reactors were reduced during open-circuit mode operation as compared to closed-circuit operation. For planted reactors, R'1 showed better fecal coliform removal than R'2 in both open and closed-circuit operations, suggesting the role of plants in higher fecal coliform removal.

Keywords

Integrated constructed wetland – microbial fuel cell, wastewater treatment, hydraulic retention time, microbial metagenomics, cost-benefit ratio, fecal coliforms.

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List of Abbreviations

BOD_5	5 day Biochemical oxygen demand
CBA	Cost-benefit analysis
CBR	Cost-benefit ratio
CD	Current density
CE	Coulombic efficiency
C_{mo}	Operation and maintenance cost
C_{ni}	Net investment cost
COD	Chemical oxygen demand
CPCB	Central Pollution Control Board
C_{pu}	Cost of unit power generation
C_{ti}	Total investment cost
CW	Constructed wetland
CW-MFC	Constructed wetland – microbial fuel cell
D_f	Dilution factor
DO	Dissolved oxygen
EAB	Electroactive bacteria
FC	Fecal coliform
GAC	Granular activated carbon
HRT	Hydraulic retention time
HRT	Hydraulic retention time
MFC	Microbial fuel cell
MFC	Microbial fuel cell
MPN	Most probable number
Mtoe	Million tonnes of oil equivalent
OCV	Open circuit voltage
ORP	Oxidation-reduction potential/Redox potential
PD	Power density
R_{pe}	Benefit from bioelectricity production
R_{pu}	Benefit per unit power production
Rs	Rupees
R_{tw}	Benefit from wastewater treatment
SAR	Sodium adsorption ratio
SSM	Stainless steel mesh
T	HRT in days
TC	Total coliform
TDS	Total dissolved solids
TSS	Total suspended solids
V	Effective reactor volume
VSS	Volatile suspended solids
W	Watt

Chapter 1

Introduction & Literature Review

1.1 Prologue

Water is vital for the existence of all living organisms, but this valued resource is increasingly being threatened as human populations grow and demand more high-quality water for domestic purposes and economic activities. Among the various environmental challenges that India is facing this century, freshwater scarcity ranks very high (Katyaini et al., 2020). The key hurdles to better management of the water quality in India are the temporal and spatial variation of rainfall, improper management of surface runoff, uneven geographic distribution of surface water resources, persistent droughts, overuse, and contamination of groundwater (Srikanth, 2009). India occupies 2.4% of the total land area (Kumar, 2012) and 4% of the total water resources of the world (Kaur et al., 2012). With these limited resources, our nation has to support 17.7% of the total world population according to the UN (United Nations) world population data published in February 2020. Water requirement for various sectors is given in Table 1.1.

Water, food, and energy securities are emerging as increasingly vital concerns for Indian rural sectors. Due to extensive urbanization, industrialization, and increasing agricultural demands, most of the river basins in India are now facing moderate to very severe

Table 1.1 Water Requirements of Various Sectors (A Future Projection)

Sector	**Water Demand (billion cubic meters)**		
Year	2010	2025	2050
Drinking water	56	73	102
Irrigation	688	910	1072
Industry	12	23	63
Energy	5	15	130
Others	52	72	80
Total	813	1093	1447

Source: Ministry of Water Resource (MoWR), Government of India water shortages (Sharannya et al., 2021). This water demand stress has also been transferred to the available groundwater (GW) as was seen in the recent water crisis in Tamil Nadu province in India, especially in the capital city of Chennai. The situation could be mitigated by enhancing water use efficiency and proper wastewater management. Thus, wastewater/low-quality water is emerging as a potential source for demand control after essential treatment. Effluent from the treatment plants, is often, not suitable for household reuse, and its application is mostly restricted to agricultural and industrial purposes. However, wastewater reuse is associated with higher levels of health risks especially in India and other developing countries, where the wastewater is rarely treated in rural and suburban regions, and large volumes of untreated wastewater are being disposed of in surface water bodies. In decentralized communities, the management of domestic wastewater has become a big problem, particularly with the improvement in the living standards of people. For this, sufficient capacity is not available for handling this domestic wastewater. A bulk of this wastewater is either discharged in open channels, accumulated in low-lying areas or flows through natural open drains.

1.2 Status of sewage generation and treatment in India

Together, India's largest cities generate more than 8,254 million liters per day (MLD) of sewage. Of this, less than 30 percent of what is collected undergoes treatment before it is disposed into freshwater bodies or the sea. Unfortunately, these figures exclude sewage generated in informal settlements and in smaller cities and towns where an acute lack of municipal infrastructure for water supply and sewage collection makes data hard to find (Hingorani, 2011). As per Central Pollution Control Board (CPCB) rules, a city or town's municipality or water authority is responsible for collecting and treating 100 percent of the sewage generated within its jurisdiction. The level of treatment of sewage depends on where it will be disposed of, the treatment standards are higher for disposal into freshwater bodies than the sea. Sometimes wastewater stagnates in pools from where it leaches into the groundwater table and contaminates underground aquifers. Often, informal industry and peri-urban agriculture add industrial and agricultural waste to the mix. On the one hand, untreated sewage is the single most important contributor to surface and groundwater pollution in the country. As a result water-borne diseases like diarrhea, caused by consuming fecally contaminated water, are the largest cause of child mortality in India. A study by the World Bank's Water and Sanitation Program calculates the per capita economic cost of inadequate sanitation (including mortality impact) at Rs 2,180 (HPEC, 2011). There are additional costs that are seldom valued.

For example, water pollution poses costly threats to the ecology, aquatic life, and the fishing industry. Most importantly, pollution of freshwater bodies is inextricably linked to growing water scarcity as polluted water is unsafe to use directly.

Urban water supply and sanitation are important basic needs for the improvement of the quality of life and enhancement of the productive efficiency of the people. In urban areas, water is tapped for domestic and industrial uses from rivers, streams, wells, and lakes. Almost 80% of the water supplied for domestic use, comes out as wastewater. In most cases, untreated wastewater either sinks into the ground as a potential pollutant of groundwater or is discharged into the natural drainage system causing pollution in downstream regions. In 2009, CPCB published a report regarding the status of water supply, wastewater generation, and treatment in class I cities and class II towns in India (CPCB, 2009). Municipal sewage may be defined as "waste (mostly liquid) originating from a community; may be composed of domestic wastewaters and/or industrial discharges". It is the major source of water pollution in India, particularly in and around large urban centers. In India, about 78% of the urban population has access to safe drinking water and about 38% of the urban population has access to sanitation services which is a major concern. This indicates that demand for drinking water is surging since the population in urban areas is increasing. Domestic wastewater management is one of the most pressing issues as the urbanization trend continues over the globe. Among the challenges faced by urban planners, paramount is the need to ensure ongoing basic human services such as the provision of water and sanitation. The under-management of domestic wastewater in many southern urban areas presents a major challenge. The accumulation of human waste is constant and unmanaged wastewater directly pollutes the surface and ground water.

There are 498 class I cities (population more than 1,00,000) and 410 class II towns (population 50,000-1,00,000) in India. Out of about 38000 MLD of sewage generated from class I cities and class II towns, treatment capacity exists for only about 12000 MLD i.e. only 31.57%. Thus, there is a large gap between the generation and treatment of wastewater in India. Even the existing treatment capacity is also not effectively utilized due to operation and maintenance problems. Operation and maintenance of existing sewage treatment plants (STPs) and sewage pumping stations are not satisfactory, as nearly 39% of plants are not conforming to the general standards prescribed under the Environmental (Protection) Rulesfor discharge into streams as per the CPCB's survey report (CPCB, 2009). In several cities, the existing treatment capacity remains underutilized while a lot of sewage is discharged without treatment in the same city. An auxiliary power backup facility is required at all the intermediate & main pumping stations of all the STPs, which is currently nonexistent. Figure 1.1 illustrates the amount of wastewater generated in metropolitan, class I, and class II cities of India and the treatment capacity.

1.2.1 Status of sewage generation and treatment in class I cities & class II towns

As per the report published by the Central Pollution Control Board (CPCB, 2008), 93% of total wastewater in India is generated from 498 class I cities. The total Sewage treatment Capacity of class I cities is reported at around 11,554 MLD, which is 32% of the sewage generation (35558 MLD). Table 1.2 shows the state-wise sewage generation in class I cities in India. The total wastewater generated in class II towns is approximately 2697 MLD. The total treatment capacity is nearly 234 MLD which is just 8.67% of the amount of sewage generated. Table 1.3 shows the status of sewage generation and treatment in class II towns in India. Figure 1.1 gives an illustrative comparison of the sewage treatment capacity of the metropolitan, class I, and class II cities in India. An illustrative cost analysis associated with several high-rate wastewater treatment technologies practiced in India is given in Figure 1.2.

1.2.2 Evaluation of sewage treatment plants in India: A CPCB survey

According to a report by the Central Pollution Control Board (CPCB, 2009), India, the major contributors to wastewater are the states of Maharashtra, Uttar Pradesh, West Bengal, Delhi, and Gujarat. A large number of the cities/towns in these states either do not have any sewerage system or it is overloaded or defunct. The existing sewer systems in many towns, cities, and urban regions are not at all appreciable. The wastewater treatment facilities in these states do not perform properly due to poor design, poor maintenance, and unskilled approaches. Since there is a large gap between domestic wastewater generation and treatment in India, it leads to the most important reason for surface water and groundwater pollution. Besides the inadequacy of the treatment capacity, the poor functional status of the STPs is also a major contributing factor. Under the centrally funded National River Action Plan or The Ganga Action Plan; several wastewater treatment plants have been established but the initiative has become futile due to the lack of proper operation and maintenance. In 2008, CPCB published a report evaluating the status of the operation and maintenance of STPs in India. The detail of the received information is shown in Table 1.4.

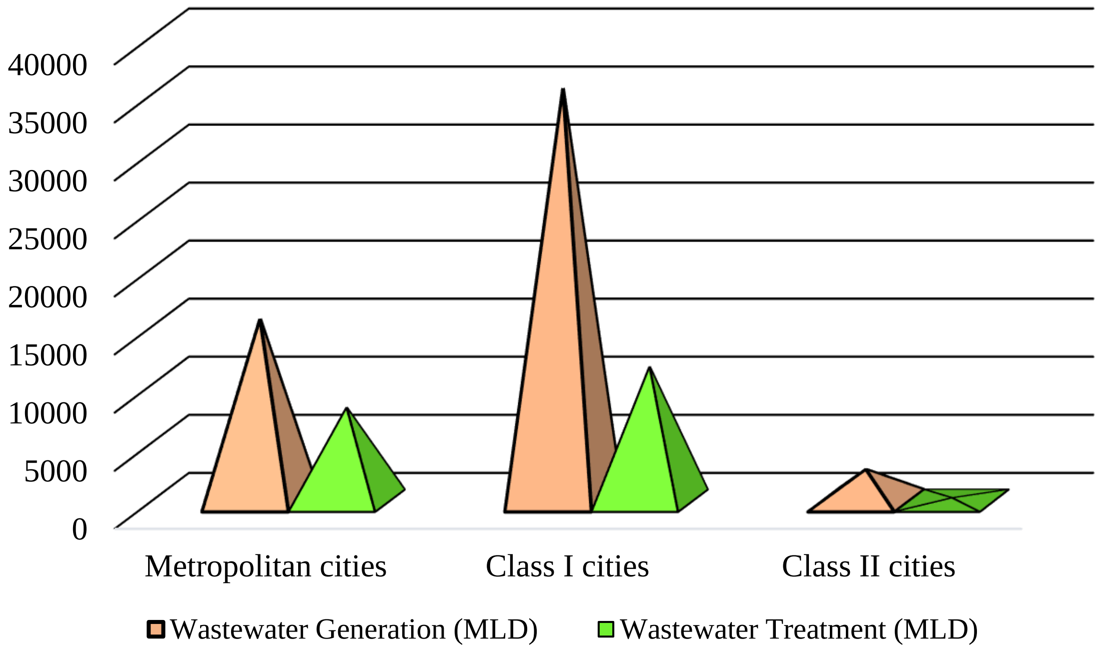

Fig. 1.1 Comparison of wastewater generation and treatment in metropolitan, class I, and class II cities in India

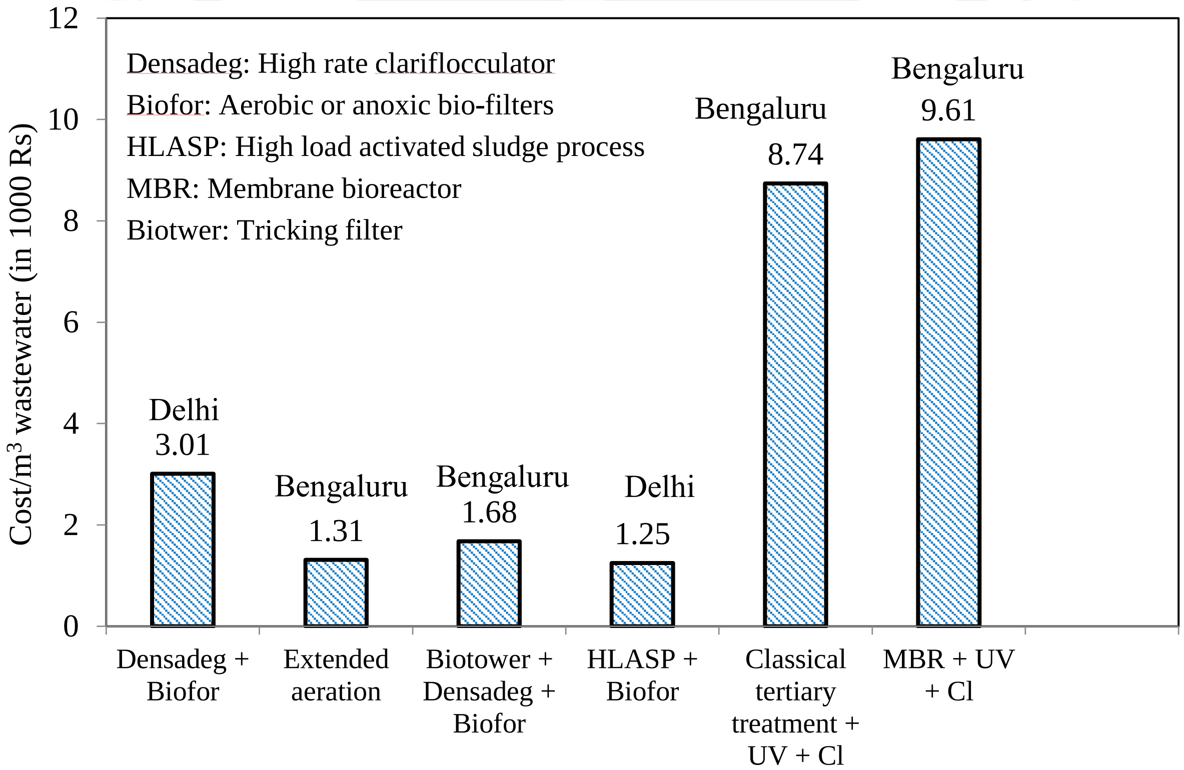

Fig. 1.2 Cost of different high-rate wastewater treatment technologies practiced in India

This survey by CPCB revealed shocking technological and managerial problems in the operation and maintenance of STPs. A total of 175 STPs were identified by CPCB over 15 states. The state-wise distribution is shown in Figure 1.3. Out of these 175 STPs, information was received from only 79 STPs in the prescribed format. In most STPs, the capacity utilization was found to be adequate and the most neglected aspect in the operation of STPs is the daily monitoring and supervision. Most of the plants lack an alternate power supply and the valorization of biogas generated from anaerobic units was found inadequate. The primary derelict area of STP operation was the sludge handling. 43 STPs, having activated sludge process (ASP) or other high-rate aerated systems were found to be with an out-of-order sludge handling facility. Many STPs (67%) were found to be running in overloaded conditions. 28 STPs were being operated with a stabilization pond system. Among them in 24 STPs the cleaning of accumulated sludge was irregular. Despite having anaerobic digesters and anaerobic reactors, there was no gas generation and utilization in 13 plants.

It was observed that in most cases the utilization of the biogas generated from UASB (UP flow anaerobic sludge blanket) reactors and sludge digesters was inadequate. In 14 STPs the gas generated from anaerobic units is not utilized and just flared. The generated gas was utilized in only 12 STPs as domestic fuel, generator fuel, or fuel for gas engines. Only 13 STPs were equipped with operational alternative power supply facilities. Six STPs were equipped with an alternate power supply facility but were unable to utilize it due to financial constraints.

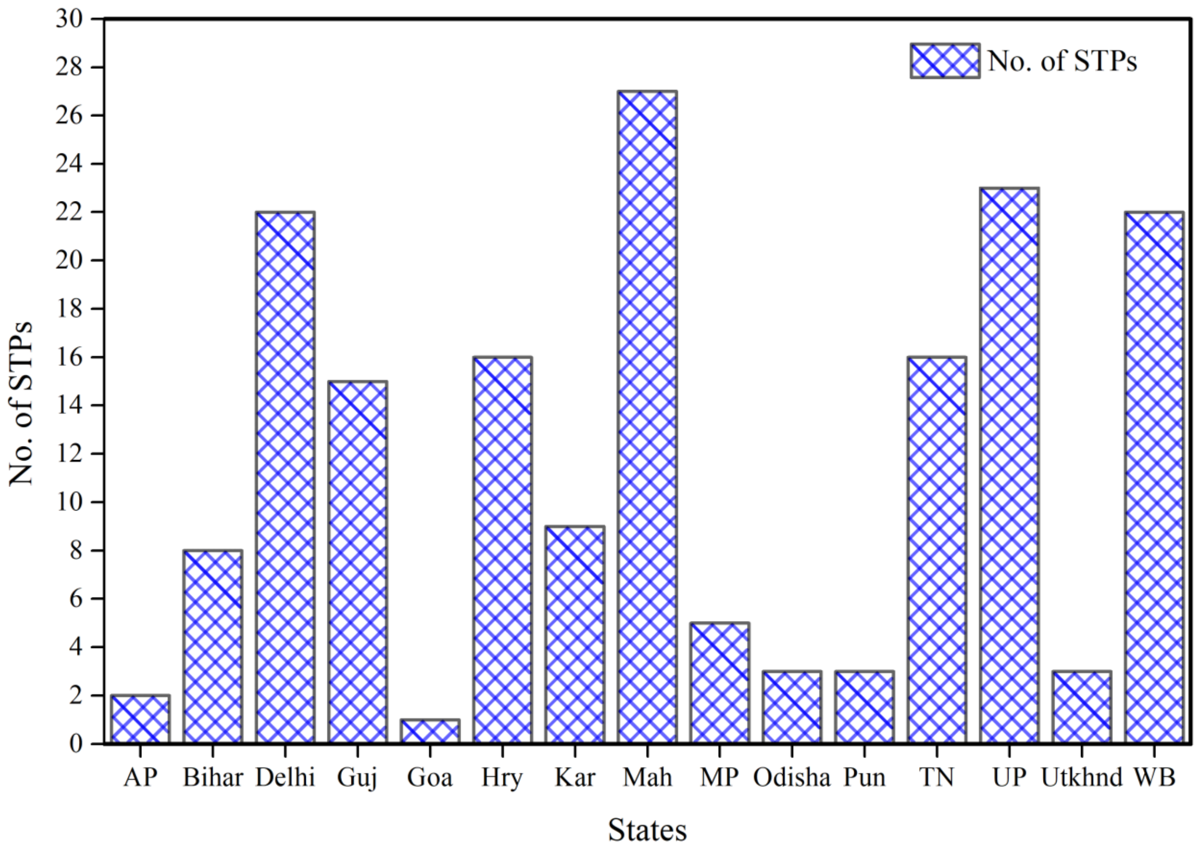

Fig. 1.3 Statewise distribution of STPs in India (see Table 1.4 for abbreviation)

Table 1.2 Sewage generation and treatment status in Class I Cities (State-wise)

Sl. No.	State or Union Territory	No. of class I cities	Sewage generation (MLD)	Sewage Treatment capacity (MLD)	% Treatment capacity
1	Andaman & Nicobar	1	12.00	-	-
2	Andhra Pradesh	47	1760.6	654.00	37.14
3	Assam	5	380.14	-	-
4	Bihar	23	1009.7	135.5	13.41
5	Chandigarh	1	429.76	164.79	38.34
6	Chhattisgarh	7	350.47	69.00	19.68
7	Delhi	1	3800.0	2330.0	61.31
8	Goa	1	9.79	-	-
9	Gujarat	28	1680.92	782.5	46.55
10	Haryana	20	626.69	312.00	49.78
11	Himachal Pradesh	1	28.94	35.63	100
12	Jammu and Kashmir	2	213.93	-	-
13	Jharkhand	14	830.47	-	-
14	Karnataka	33	1790.4	43.44	2.42
15	Kerala	8	575.17	-	-
16	Madhya Pradesh	25	1248.72	186.1	14.9
17	Maharashtra	50	9986.29	4225.25	42.31
18	Manipur	1	26.74	-	-
19	Meghalaya	1	20.84	-	-
20	Mizoram	1	5.71	-	-
21	Nagaland	1	13.62	-	-
22	Odisha	12	660.73	53.00	8.02
23	Puducherry	2	56.46	-	-
24	Punjab	19	1528.26	411.00	26.89
25	Rajasthan	24	1382.37	54.00	3.9
26	Tamil Nadu	42	1077.21	333.42	30.95
27	Tripura	1	24.00	-	-
28	Uttar Pradesh	61	3506.01	1240.13	35.37
29	Uttarakhand	6	176.97	18.00	10.17
30	West Bengal	60	2345.21	505.92	21.57

Source: Status of sewage treatment in India, CPCB (CUPS/61/2005-06) and questionnaire survey 2007, (-) means no information was received against the survey.

Table 1.3 Status of sewage generation and treatment in Class II towns

Sl. No.	State or Union Territory	No. of class II towns	Sewage generation (MLD)	Sewage Treatment capacity (MLD)	Treatment capacity (%)
1	Andhra Pradesh	52	217.59	10.42	4.78
2	Assam	08	6.46	-	-
3	Bihar	14	107.42	2.00	1.86
4	Chhattisgarh	07	40.82	-	-
5	Goa	02	13.89	18.18	100
6	Gujarat	31	227.55	-	-
7	Haryana	07	43.52	-	-
8	Jammu and Kashmir	04	27.86	-	-
9	Jharkhand	10	78.21	-	-
10	Karnataka	26	233.37	12.18	5.21
11	Kerala	26	231.32	-	-
12	Madhya Pradesh	23	130.9	9.00	6.80
13	Maharashtra	34	213.73	29.00	13.56
14	Meghalaya	01	11.25	-	-
15	Nagaland	01	1.36	-	-
16	Odisha	12	78.42	-	-
17	Puducherry	01	7.98	-	-
18	Punjab	14	157.40	42.80	27.19
19	Rajasthan	21	147.79	-	-
20	Tamil Nadu	42	184.67	29.30	15.86
21	Uttar Pradesh	46	345.70	12.61	3.64
22	Uttarakhand	01	9.07	6.33	69.79
23	West Bengal	27	180.42	61.88	34.29

Source: Status of sewage treatment in India, CPCB (CUPS/61/2005-06) and questionnaire survey 2007, (-) means no information was received against the survey.

Table 1.4 Summary of CPCB survey report on sewage treatment plants in India

Sl. No.	State	No. of STPs identified	Information received from the no. of STPs	No. of STPs visited	Remarks
1	Andhra Pradesh (AP)	2	-	-	
2	Bihar	8	6	4	
3	Delhi	22	19	16	Includes micro STPs
4	Gujarat (Guj)	15	12	-	
5	Goa	1	1	1	
6	Haryana (Hry)	16	14	6	
7	Karnataka (Kar)	9	7	-	
8	Maharashtra (Mah)	27	-	13	Includes Powai Lake, Mumbai
9	Madhya Pradesh (MP)	5	1	-	
10	Odisha	3	-	-	
11	Punjab (Pun)	3	12	-	
12	Tamil Nadu (TN)	16	4	6	
13	Uttar Pradesh (UP)	23	3	14	
14	Uttarakhand (Utkhnd)	3	-	2	
15	West Bengal (WB)	22	-	8	Includes CETP (Kolkata)
	TOTAL	**175**	**79**	**68**	

(-) means no information was available in the report

1.2.3 Decentralized wastewater treatment is the need of the hour

India is a growing economy and economic growth indicates increased water use and consequent wastewater generation. As is seen in the previous discussion central sewage treatment plants have failed to be established as a sustainable solution for the problem of wastewater treatment and reuse, it is very highly advisable and necessary to treat the wastewater on the community level at the site of generation before disposal (Philip et al., 2019; Eriksson et al., 2002). The general approach of this decentralized wastewater treatment scheme (DWWT) is sustainable wastewater treatment and reuse to minimize freshwater utilization. Many researchers have suggested transferring the disposal-based linear STP systems to recovery-based cyclic closed-loop systems that can promote water conversation, increase nutrient utilization, and contribute to public health (Philip et al., 2019). This DWWT system can be more flexible and can adapt easily to local conditions and most importantly it can grow with the community population (Jhansi and Mishra, 2013). In particular for small urban towns, decentralized systems are considered more suitable than centralized wastewater treatment. Some of the typical advantages attributed to decentralized systems are that they can be installed without requiring a huge budget, especially at isolated locations, or that they save the money required for a sewerage network and increase the possibility of reuse of treated water without extra expenditure required for the water supply network (Wang, 2014; Massoud et al., 2009).

Technically the aerobic treatment systems are capable of producing the most supreme quality effluents that can meet the discharge standards (Chan et al., 2009). The anaerobic systems are advantageous in terms of the scope of energy harvesting but their efficiency is less than aerobic processes (Alexiou and Mara, 2003; Melidis et al., 2009). In economic aspects, there are two types of costs associated with treatment systems (i) capital cost and (ii) operation and maintenance (O&M) cost. The capital cost mainly includes the cost of design, construction, and land acquisition. In general, the total cost is generally proportional to the size of the community. The aerobic and advanced aerobic systems are more cost-intensive than anaerobic systems as the O&M cost increases due to power requirement charges. The main focus of conventional engineered wastewater treatment technologies is on electro-mechanical solutions which demand high capital cost and require a significant amount of O&M cost. Besides, these systems have shorter life cycles than many natural treatment schemes which can produce safe water for reuse (Singh et al., 2015). Innovative decentralized/on-site wastewater treatment plants aiming not only at treating the wastewater but also providing other benefits such as the reuse of water, energy reuse, or nutrient reuse—depending on the local context—are the need of the day. Thus, wastewater reuse and recycling may be highly sustainable because reclaimed water, in addition to increasing its availability, can be fit-for-use and therefore avoid costly over-treatments (Rodriguez et al., 2009; Daigger, 2009). This means that it fulfills the requirements of the end users. In the case of India, taking into account that there is a low ratio of wastewater treatment, it can be beneficial in two ways: on one hand, it can lead to higher wastewater treatment ratios, and on the other, it can mitigate water scarcity scenarios. However, socio-cultural, economic, and regional aspects have to be taken into account while selecting the technologies, to make the process sustainable (Brunner et al., 2018).

1.3 An overview of constructed wetland technology

Conventional wastewater treatment technologies like activated sludge process and membrane separation are costly energy-intensive and not recommended in rural areas. Moreover, they are not able to meet the stringent water and wastewater standards set by the various governments (Wu et al., 2014). Thus, selecting a low-cost and effective wastewater treatment system is vital in developing countries like India. Constructed wetland (CW) is a green technology to treat various types of wastewater and has gained popularity over the last decade, owing to its low cost and low energy requirements (Wu et al., 2015b). These systems, mainly comprised of vegetation, substrates, soils, microorganisms, and water; utilize physical, chemical, and biological mechanisms to remove various contaminants or improve the water quality (Vymazal, 2011). These mechanisms are broadly classified into biotic and abiotic processes. The biotic processes include microbial degradation, plant uptake, biotransformation, etc. and the abiotic processes include sedimentation, hydrolysis, photolysis, oxidation, adsorption, absorption, etc.

CWs are mainly classified into two categories based on wetland hydrology, free water surface constructed wetlands (FWSCW) and subsurface flow constructed wetlands (SSFCW). FWSCWs are almost similar to natural wetlands, with the shallow flow of wastewater over-saturated media. Whereas in SSFCWs, wastewater flows horizontally (horizontal flow constructed wetlands, HFCWs) or vertically (vertical flow constructed wetlands, VFCWs) through the media. Another wetland type, known as hybrid constructed wetlands (HCWs), is designed to exploit the advantages of individual wetland systems by the combination of different types of wetland systems. Recently, to intensify the removal processes and/or to minimize the area requirement of CWs, enhanced CWs have been proposed (artificial aerated CWs, towery hybrid CWs, step-feeding CWs, etc.) (Wu et al., 2015a). The classification of

CWs is shown in Figure 1.4. Table 1.5 summarizes some literature results highlighting the use of CWs as decentralized wastewater treatment scheme for wastewater treatment.

1.3.1 Challenges of constructed wetlands as a decentralized treatment scheme

CWs have some intrinsic drawbacks that can limit their application and long-term stability. The performance of CWs is largely dependent on the ambient temperature fluctuations during seasonal variations. Generally, the pollutant removal capacity of CWs is reduced in the winter as compared to summer or autumn. This trend is particularly noticeable in the case of nutrient removal (nitrogen and phosphorus). Substrate clogging is another big concern especially when CWs are used in wastewater treatment with high organics and suspended solids loading rates (Ruiz et al., 2010). The prevalence of the anaerobic zone at the bottom of CW reactors reduces the rate of microbial decomposition which leads to an enhanced areal footprint. This increases the capital cost of reactor installation in the rural and suburban regions. Microbial assemblage is a key factor for the organics and nutrient removal performance of CWs, which could be susceptible to toxic biorefractory components of raw domestic or industrial wastewater. Thus it is important to focus on the long-term reactor operation using raw domestic wastewater to get an idea of the applicability of CWs on real term scenarios. In majority of the studies related to domestic wastewater treatment, coliform removal was not focused.

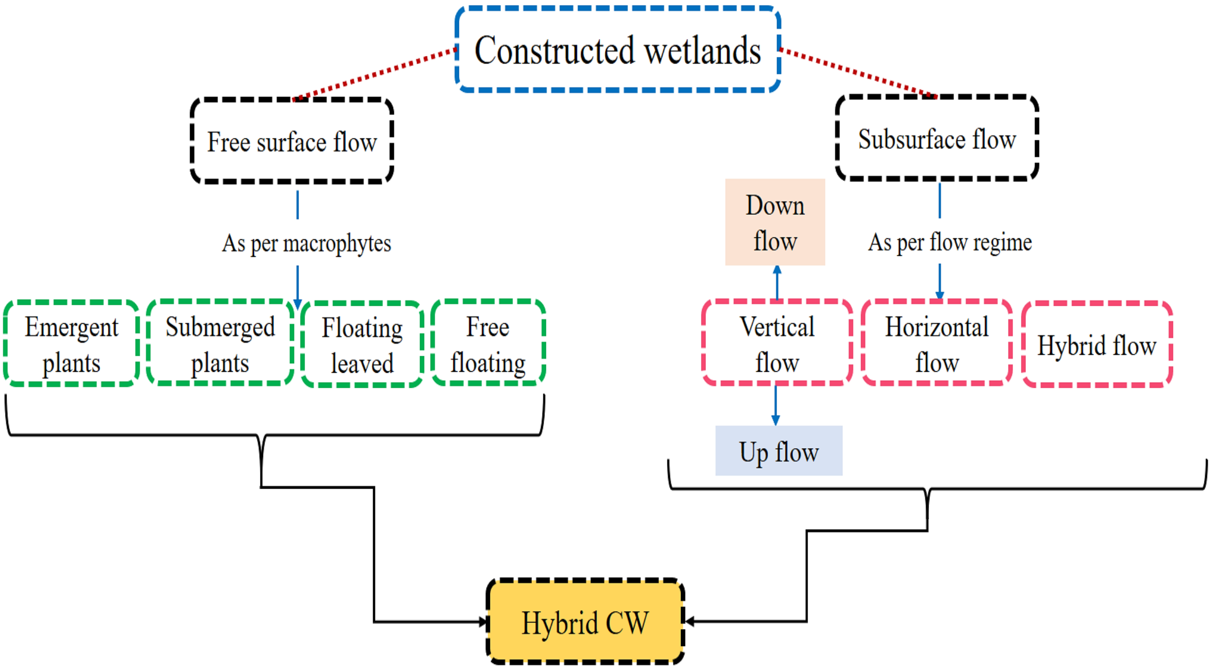

Fig. 1.4 Classification of constructed wetlands

Table 1.5 Studies summarizing the use of constructed wetlands as a decentralized treatment scheme

Objectives and reactor details	Wastewater characteristics	Observations and limitation	Reference
Domestic wastewater treatment in vertical flow CW. Surface area 24.5 m^2 (3.5x7) and planted with *Heliconia psittacorum.*	COD: 564 mg/L BOD_5: 282 mg/L NH_4^+-N: 57 mg/L TS: 731 mg/L	74% COD removal, 84% TSS removal, and 81.5% BOD removal. NH_4^+-N removal 58%. Limitation: High surface area was required, coliform removal not investigated.	(Decezaro et al., 2018)
Decentralized wastewater treatment via VFCW (0.6x0.4x0.39 m) with and without supplementary aeration. Planted *Canna indica.*	DO: 1.6 mg/L TSS: 60 mg/L COD: 82 mg/L TN: 14.4 mg/L	The optimal HRT (42h) for the aerated unit was less than the non-aerated unit (48h). TSS, COD, and TN removal were 81.2%, 85%, and 89.9%. Limitation: Coliform removal not reported. Aeration was necessary to reduce HRT.	(Zhu et al., 2013)
Treatment of domestic wastewater with HFCW (8x2x1m) planted with *Phragmites australis* for irrigation reuse.	DO: 0.4 mg/L BOD_5: 122 mg/L TSS: 68.95 mg/L TN: 121.23 mg/L	Satisfactory sewage treatment in CW with HRT 3 d winter. Limitation: Tertiary treatment was necessary before reusing due to existing pathogenic microorganisms in the effluent.	(Ham et al., 2007)
Medium-strength wastewater treatment in a pilot scale fish farm with integrated identical HFCW and VFCW (0.24x0.9x0.45m). *Phalaris arundinacaea L* was used as the wetland macrophyte.	COD: 172 mg/L DO: 0.7 mg/L NH_4^+-N: 50.9 mg/L TP: 25.4 mg/L	Considerable removal of COD (84%) and NH_4^+-N (93%) was observed with an HRT of 3.4 d. Limitation: Two separate units were applied that increased the areal footprint.	(Behrends et al., 2007)

Table 1.5 continued

Treatment of septic tank effluent in HFCW (area 6.8 m^2 per population equivalent and 0.7m deep), planted with *Zizaniopsis bonariensis* and coated with waterproof blankets to prevent groundwater pollution.	COD: 584 mg/L TSS: 305 mg/L TN: 48.5 mg/L TP: 25 mg/L	Initially, the COD removal was 54% and it increased to 98% in the next 10 years. Media clogging was observed after 4 years. Removal efficiency for TSS, TN, and TP was 55.33%, 71%, and 26.5% respectively. Limitation: Coliform removal not reported.	(Phillipi et al., 2006)
Removal of emerging pollutants like trimethyl amine (TMA), propylene glycol (PG), and sodium dodecyl sulfate (SDS) from greywater with baffled CW (10x2.55x1.2m) planted with *Phragmites australis*.	COD: 228 mg/L SDS: 25.5 mg/L PG: 29 mg/L TMA: 12.2 mg/L	Wetland performance was significantly affected by water temperature and seasonal variation. Removal of PG and TMA was 90% and 92% respectively with 12.5 d HRT. SDS removal was 96% with 16.5 d HRT compared to 86% with 12.5 d HRT. COD reduction was 92-95%. Limitation: Synthetic wastewater was used instead of raw wastewater.	(Dash, 2013)
Economical Greywater treatment with easily maintainable VFCW (0.95x0.95x0.55m) and reuse for landscape irrigation. The VFCW was planted with *Lactuca sativa*.	COD: 839 mg/L TSS: 158 mg/L TN: 34.3 mg/L TP: 22.8 mg/L	Maintaining HRT of 8 h the removal of COD, TSS, TN, and TP were 81%, 98%, 69%, and 71%. Fecal coliform concentration was dropped by three to four orders. Limitation: Seasonal performance variation in long-term wetland operation was not reported.	(Ruiz et al., 2010)

DO: dissolved oxygen, HFCW: horizontal flow constructed wetland, HLR: hydraulic loading rate, HRT: hydraulic retention time, TN: total nitrogen, TP: total phosphorus, TS: total solids, TSS: total suspended solids, VFCW: vertical flow constructed wetland of low oxygen transfer or denitrification due to low amounts of available organics (Wu et al., 2014), especially under high nitrogen loading rates. Furthermore, some recalcitrant pollutants and heavy metals in industrial wastewater also present challenges to the performance of CWs (Herrera et al., 2008).

1.3.2 Constructed wetlands and technology integration

With the deteriorating environment and stringent discharge standards, including the emphasis on effluent reuse, CW systems are unable to cope with the updated guidelines (Winward et al., 2008), despite improvements in design and operational strategies. Therefore, combining or integrating CWs with other existent or emerging technologies, such as membrane bio-reactor (MBR) (Mutamim et al., 2012), electrochemical oxidation (Anglada et al., 2010), microbial fuel cells (MFCs) (Mohan et al., 2008), etc. have emerged in recent years to maximize the individual advantages in terms of wastewater treatment. These technologies have been proven to be robust in the treatment of specific pollutants and/or as green processes for energy recovery although they still have some limitations, as documented in the literature (Dalmau et al., 2015; Escapa et al., 2015). Along with performance enhancement, the incorporation of technologies may serve some other purposes like promoting organics and nutrient removal, elimination of refractory organics and metallic pollutants, and energy recovery, etc. (Liu et al., 2015).

Guo et al. (2014), proposed an intensified process of coupling sequencing batch bio-film reactor technology (SBBR) with CW to treat municipal wastewater and achieved overall COD, NH_4^+, TN (total nitrogen), and TP (total phosphorus) removal of 97%, 98.5%, 91.5%, and 88.5%, respectively. In another study, Li et al. (2004), proposed a novel process for greywater treatment by CWs in combination with TiO2-based photocatalytic oxidation for suburban and rural areas without a sewer system. With the combined photocatalytic process, the TOC in the effluent of CWs could be further reduced from 10 mg/L to 1 mg/L. Furthermore, the total coliform after the photocatalytic process was only 14/100 mL, satisfying the European bathing water quality as well. Horn et al. (2014), successfully treated university sewage water with photocatalytic oxidation and CW coupling.

1.4 Microbial electrochemical technology and CW-MFC integration

Microbial electrochemical technology (MET) is also known as bioelectrochemical systems (BES), which is based on microbial interaction with insoluble electron donors and acceptors. This technology mostly relies on metabolic electron exchange that is, electrons which are removed from the donor and supplied to an electron acceptor (solid state electrode) through an electroconductive material (Rabaey et al., 2007; Rosenbaum and Franks, 2014). MET has also been used for many environmental remedial purposes such as wastewater treatment, desalination, and soil bioremediation, etc. (Arends and Verstraete, 2012). The very first study on MET was regarding substrate oxidation by *Escherichia coli*, with subsequent bioelectricity generation. The first effort to nomenclate this technology into different classifications was documented by Schröder et al. (2015). The application of microbe-driven electrochemical processes for complex organic production, energy generation, and environmental remediation is called microbial electrochemical technology or MET. Depending on the operating conditions, they can be classified as primary or secondary MET. The classification flow chart is shown in **Figure 1.5** In primary MET, the microbial electrochemical processes either involve extracellular electron transfer (EET) mechanisms directly from cell to acceptor or are mediated by electron shuttles. In the secondary MET, the bio-electrochemical processes are controlled by the adjustment of the microbial environmental conditions (pH, oxygen, metabolite concentrations, etc.), as in microbial electrolysis of hydrogen or soil remediation. Roughly, MET can be classified as power producers (electrons derived from oxidized organic matter are conducted to the cathode via an external circuit), power consumers (consumption of external power to achieve bio-electrochemical cathodic reactions due to small or negative potential differences not allowing electron flow from anode to cathode) and as intermediate systems that neither produce nor consume power, but require stable electrochemical conditions (Ramírez-Vargas et al., 2018).

The rate of electrochemical reaction can be enhanced by microbial electrocatalysis. This is mediated by microbial extracellular electron transfer. This electrocatalysis is used in microbial fuel cells (MFC) for subsequent wastewater treatment and energy generation. Due to the potential of MET for simultaneous wastewater treatment and energy generation, different studies have explored the technical viability of combining this technology with CWs since 2012. CWs are extensively investigated technology for the treatment of several types of wastewater like domestic, industrial, acid mine drainage, agricultural runoff, etc. (Vymazal, 2009). To reduce the surface requirements of decentralized treatment units, CWs have been converted into intensified integrated systems, including multiple design modifications. Amidst which the latest is the combination of MET with CW (Xu et al., 2016).

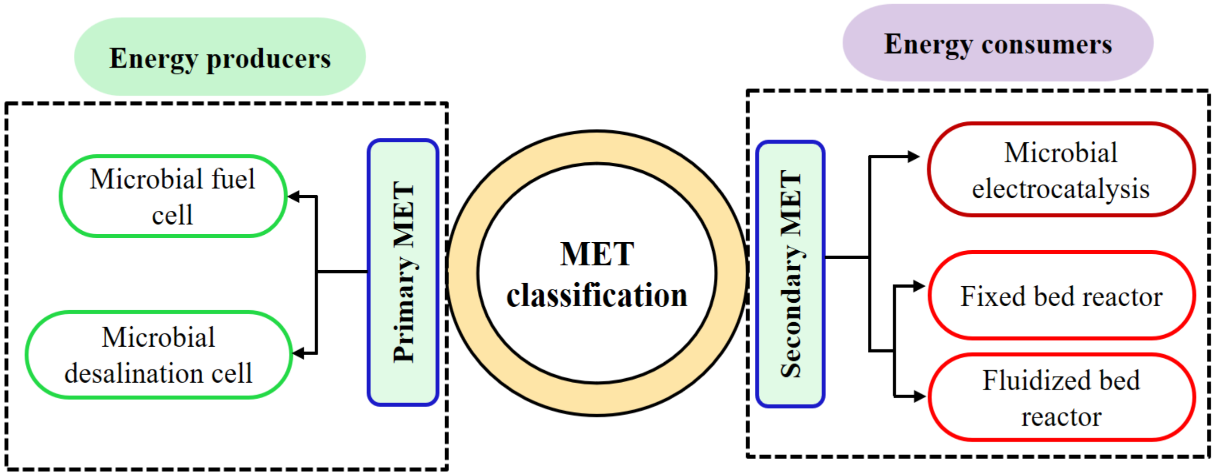

Fig. 1.5 Classification of MET technology proposed by Ramirez-Vargas et al. (2018).

1.4.1 Microbial extracellular electron transfer

In an MET, the favorable consortia between fermentative and electroactive bacteria (EAB) are achieved on the predominance of the anaerobic environment within an aqueous medium. The role of the fermentative bacteria is to break down the complex organic compounds into simpler organic structures like volatile fatty acids, cellulose, etc. which are easily oxidized by the electroactive bacteria. The metabolic characteristics of the EABs allow them to obtain energy by transferring the electrons to nearby electron acceptors characterized as insoluble extracellular conductive material. These phenomena promote the development of these microorganisms in such an environment where the growth is limited to the availability of electron acceptors or electron donors (Xu et al., 2016; Logan and Rabaey, 2012; Reimers et al., 2001). The establishment of bacterial colonies leads to the formation of biofilms on the solid insoluble electrodes. This biofilm plays an important role in electron transfer. Extracellular electron transfer (EET) by bacteria can be affected by the potential difference between the electrode and the electron carrier. The transfer of electrons is executed in two separate ways (i) direct extracellular electron transfer (DEET) and (ii) mediated extracellular electron transfer (MEET). The complete understanding of the dynamics of the electron transfer between the EAB and the electron acceptor is still being studied (Bonanni et al., 2012; Butti et al., 2016).

1.4.2 Direct extracellular electron transfer or DEET

DEET requires physical contact between the electrode surface and the microbes which are usually attached via the formation of an electroactive biofilm. In this way, the microbes present in the first monolayer would attach to the anode and transfer the electrons. This makes the microbial cell density a limiting factor in catalysis (Mao and Verwoerd, 2013). This process is shown in Figure 1.6 (a) and is represented by equation 1.1. Some EAB species can also generate nanowires (also known as electro-conductive pili) to reach the distant anode or to interconnect among several biofilm layers as shown in Figure 1.6 (b). These nanowires are vesicular extensions of the periplasm and outer cell membrane and are generally 2 – 3 µm in length. These pili facilitate the direct electron transfer between the bacteria cell and the anode. The strategy of generating the electro-conductive pili makes the thick bio-layer formation possible as well as reaching the distant electron acceptor (Patil et al., 2012). Microbes also can use other bacterial cells as terminal electron acceptors by establishing an electrical contact between two cells via nanowires. This is called direct interspecies electron transfer (DIET), as shown in Figure 1.6 (c).

$$\textit{Organic Substrates} \longrightarrow CO_2 + H^+ + e^- \tag{1.1}$$

1.4.3 Mediated extracellular electron transfer or MEET

Microbes that are not able to use either DEET or DIET have developed a separate mechanism that is mediated by electron shuttles for extracellular electron transfer and is called MEET as shown in equation 1.2. Particular microorganisms, such as *Escherichia coli*, *Pseudomonas*, *Proteus*, and *Bacillus* can naturally synthesize and excrete endogenous redox-active molecules that function as electron mediators, such as flavins or phenazine compounds (Voordeckers et al., 2010). These mediators can also be externally added or may naturally be present in the environment as humic substances (Kotloski and Gralnick, 2013). The mediators (in oxidized form) collect electrons from inside the bacteria cell or from the outer membrane become reduced and subsequently reoxidized after the transfer of electrons to the terminal electron acceptor. MEET relies on the production of their secondary metabolites by bacteria (as shown in Figure 1.7) which are low-molecular shuttling compounds like phenazines, pyocyanine, ACNQ (2 amino-3-carboxy-1, 4-naphthol-quinone), etc. Despite the reuse possibility as an electron carrier, their synthesis is energy intensive and will be suitable for closed environments with a minimal substrate replacement as in batch set-ups. However, in the case of dynamic environments like wastewater treatment, those secondary compounds will be exhausted. Some examples of bacteria that can produce their mediators to increase the MEET rate are *Pseudomonas*, and *Shewanella putrefaciens*, (Hernandez et al., 2004).

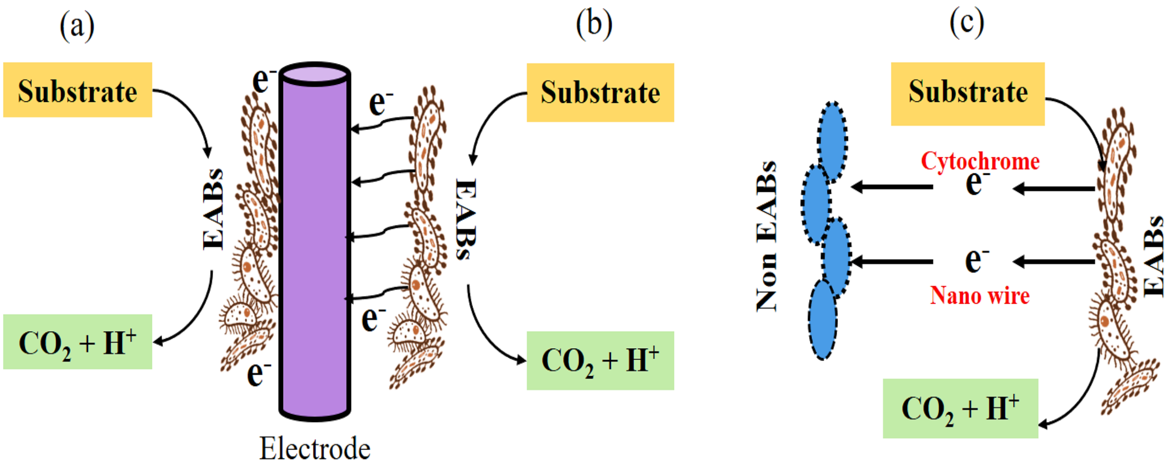

Fig. 1.6 Schematic illustration of DEET mechanism (a) transfer via attached biomass (b) transfer via nanowires (c) direct inter-species transfer via cytochrome and nanowire.

The MEET process may also take place via primary metabolites which are derived from anaerobic respiration and fermentation processes, however, this is an inefficient transfer mechanism given the slow rate of reaction. In anaerobic respiration, sulfide is formed due to sulfate reduction which gets oxidized again by interacting with available electron acceptors (iron oxides or electrodes) thereby allowing the electron transfer. In the fermentation process reduced metabolites such as hydrogen, ethanol, ammonia, etc. can be oxidized by microbial metabolism (Lovley, 2008).

$$\textit{Organic Substrates} \longrightarrow \textit{Redox mediators} \longrightarrow CO_2 + H^+ + e^- \tag{1.2}$$

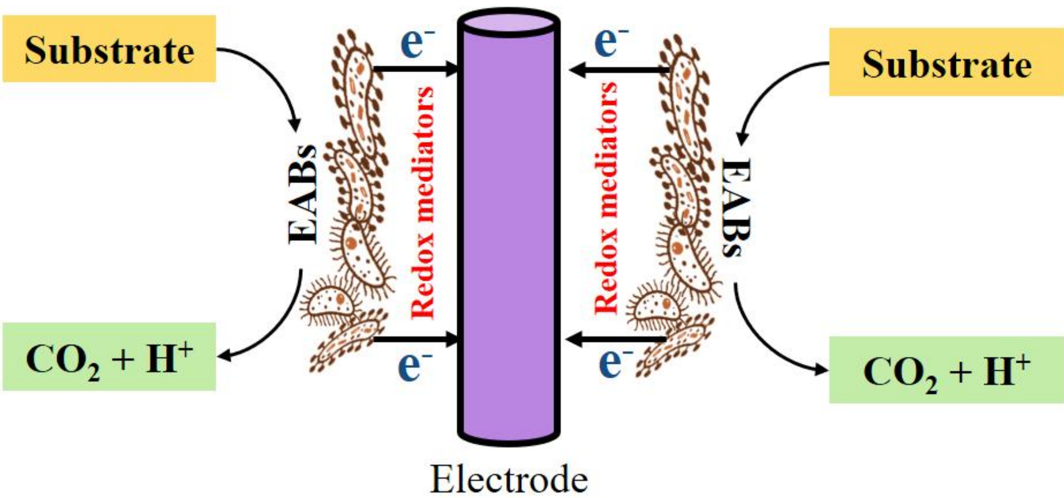

Fig. 1.7 Mediated extracellular electron transfer by secondary metabolites

1.4.4 MET for wastewater treatment

Wastewater contains a large amount of potential energy in the form of biodegradable organic matter. The energy contained in wastewater cannot be correlated directly with COD values, but the energy estimation point is 17.7 to 28.7 kJ/g COD (Heidrich et al., 2011). In the United States, wastewater treatment facilities consume around 15 GW of power (3% of nationwide power production), whereas 17 GW can be contained in wastewater of different origins, an amount that could supply the energy requirements for their treatment (Logan and Rabaey, 2012). MET have shown a potential for wastewater treatment, since their COD removal rates can reach up to 90%, with coulombic efficiencies (fraction of electrons recovered as current versus the maximum possible recovery) higher than 80% (Rahimnejad et al., 2015). It is estimated that in anaerobic digestion 1 kg COD can be converted to 4.16 kWh of power (1 kWh of usable energy in the form of electricity); therefore, if MET is intended to compete as an alternative wastewater treatment technology with simultaneous energy generation, it must reach a similar substrate conversion rate. Currently, it is not possible to produce cost-effective energy using MET. However, it constitutes an alternative technology to harvest energy directly from wastewater. Some of the limitations of the field application of METs include installation costs, expensive electrode materials, and low energy density.

From the wastewater perspective, MET systems arise as an interesting alternative, since they are easily capable of oxidizing degradable organics. METs are flexible platforms that can utilize oxidation and reduction processes to treat wastewater at a rate of 7.1 kg COD/m^3 (reactor volume/day) and offer the advantage of saving costs for aeration and sludge disposal (Wang and Ren, 2013). Wastewater treatment using MET can be accomplished by coupling a microbial anode with the cathode that carries out the reduction reaction. By completing this electrical connection between these electrodes, the flow of electrons is promoted from the anode to the cathode.

1.5 Constructed Wetland Microbial Fuel Cell (CW-MFC) Coupling

In general, there is a natural occurrence of redox gradient along the depth of a CW. This is the primary reason in favor of coupling MFC with CWs. This naturally occurring redox gradient with an aerobic zone at the water-air interface and an anaerobic zone at the bottom fulfills one basic need of MFCs for operating thereby facilitating the coupling of MFC with CW (Corbella et al., 2014). A CW-MFC consists of an anode and a cathode. The cathode is installed in the aerobic zone and the anode in the anaerobic zone. In the anodic zone or anaerobic zone, the electroactive microorganisms consume the organic matter present in the wastewater for their metabolic activity and release electrons. The microbial species associated with this electron transfer are *Geobacter sp.*, *Desulfuromonas*, *Proteiniphilum*, etc. These electrons are transferred to the anode. From the anode, the electrons flow through the external circuit and reach the cathode. The cathode is generally the aerobic zone or anoxic zone where electron acceptors like oxygen (O_2) and nitrate (NO_3) are present. These are used to complete the reduction reaction in the cathode chamber thereby completing the external circuit. Electric power is generated in the external circuit which is associated with a resistor. A conceptual illustration of a CW-MFC is shown in Figure 1.8. In the initial stages of CW-MFC development glass wool was used by some researchers to replace the proton exchange membrane for maintaining redox gradient (Yadav et al., 2012b; Zhao et al., 2013). However, researchers in later stages omitted the separator as it was not found necessary for maintaining the gradient in up-flow reactors with buried anode and cathode at the surface (Fang et al., 2013). The main objective of the CW-MFC system is to achieve high treatment performance with the added value of energy harvesting by the metabolic activity of the electroactive bacteria (EAB).

Focusing only on the removal of pollutants and not on the power generation, an innovative development has been proposed by using electro-conductive biofilters (instead of

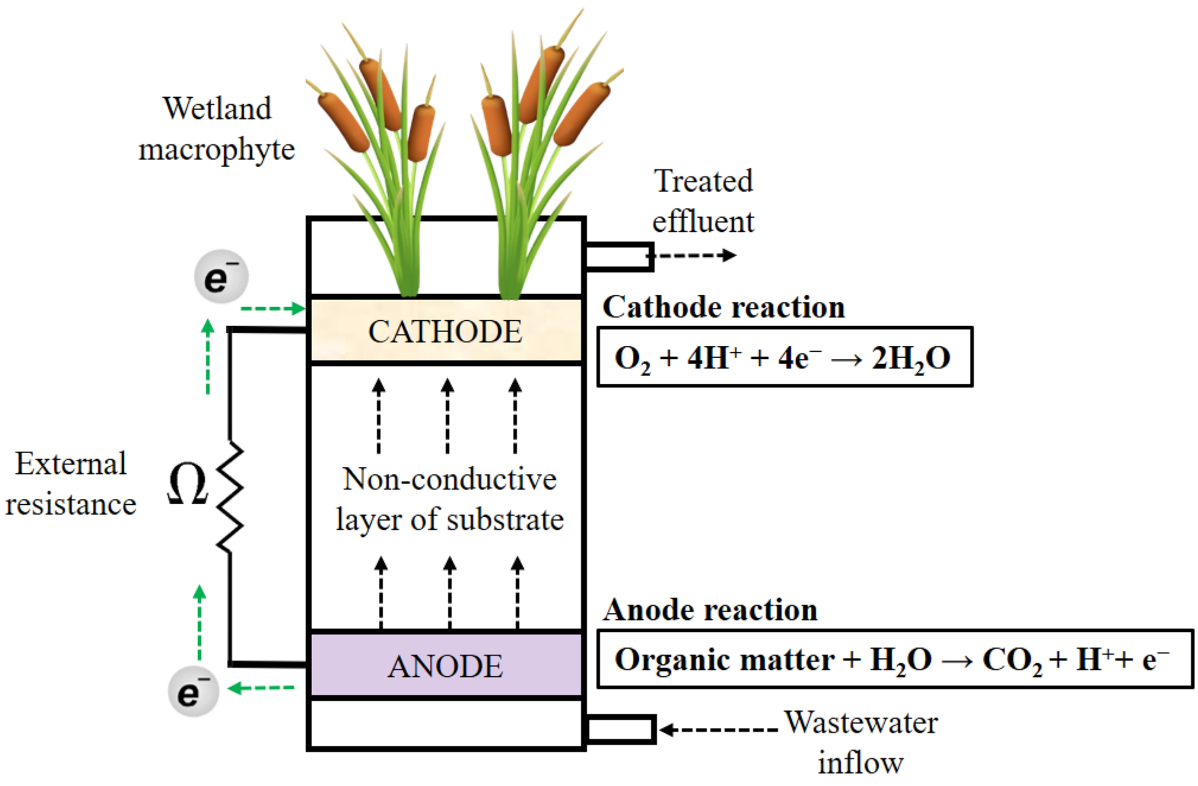

Fig. 1.8 Conceptual illustration of integrated CW-MFC system

external circuits as in the previously stated CW–MFC), resulting in the development of the microbial electrochemical-based constructed wetland (METland). In a METland system, the EABs are stimulated to generate and transfer electrons to an electro-conductive material that acts as an unlimited acceptor maximizing substrate consumption instead of leaving free electrons for methane generation, and consequently to a decrease of microbial metabolism rates (as in an anaerobic system) due to the limited number of electron acceptors.

1.5.1 Role of redox gradient

The growth of electroactive bacteria is mainly promoted by the electrical circuit. The microbes present at the anode are generally responsible for organic matter decomposition whereas in the cathode zone, the microbes work as catalysts in oxygen reduction. This reduction at the biocathode is recognized as the limiting step due to the slow reaction kinetics (Jiang et al., 2010). The concentration of the attached microbial population can be increased by enhancing the area of both electrode surfaces. In a previous study, it was reported that biomass density was increased by a factor of 8 using granular activated carbon and stainless steel mesh biocathode over carbon cloth and stainless steel mesh (Liu et al., 2014).

1.5.2 Role of Wastewater Strength

The performance of a CW-MFC system seems to be affected greatly by the COD loading or the organic loading of the wastewater. There should be an adequate amount of organics available at the anode for the electroactive bacteria to perform their metabolism. However, the amount of COD arriving at the cathode should also be kept under monitoring. If the COD loading is increased then the organic availability at the anode is increased but this also results in increased oxygen demand in the aerobic cathode chamber which can restrict the amount of oxygen available for the cathode reaction which is necessary to complete the external circuit (Freguia et al., 2008). The presence of toxic compounds or other elements may hamper the performance of CW-MFC by reducing the activity of the EABs. Researchers have reported a considerable amount of decline in power density with an increase in toxic dye concentration in the wastewater.

1.5.3 Role of Macrophyte

The main advantage of wetland plants is they exclude oxygen via the rhizosphere at the time of photosynthesis. In unplanted MFCs, the cathode is mostly placed at the air-water interface (air cathode) to increase the redox potential and efficiency of cathode reduction reactions. However, the problem with placing an air cathode is the increase in the internal resistance due to extended cathode-to-anode spacing. This problem may be overcome by using the excluded oxygen from the wetland plant rhizosphere and omitting the need to place the cathode at the air-water interface (Dong et al., 2011). The root zone of the plants can also deposit some organic matter along with oxygen and this deposition activity is enhanced in the daytime. These excluded organic matters can also increase the oxygen near the cathode and if nitrate is present in the wastewater it can help facultative denitrifying microorganisms to grow, which do not contribute to the electricity production mechanism. However if the plant roots penetrate the anode zone, anode potential fluctuation may be experienced due to the formation of aerobic microzones in the anode chamber (Timmers et al., 2010).

1.5.4 Effect of Wastewater Salinity

Though the effect of water salinity on CW-MFC performance is not tested it is hypothesized that increased salinity can result in higher current production reducing the internal resistance of the system. Previous documented studies have mentioned that salinity can affect the performance of CW and MFC in different ways (Wu et al., 2008; Yong et al., 2014). The plant species used in CWs are generally not tolerant to salinity and also many microbial species are unable to function due to high salinity (Wu et al., 2008). There are conflicting reports about the effects of salinity on MFC performance. Liu et al. (2004), reported that increasing NaCl concentrations from 5.8 to 23.4 g/L resulted in an increase in maximum power in a substrate-defined (glucose) MFC. Another study showed addition of 40 or 70 g/L NaCl solutions into the anode chambers of the MFCs resulted in complete inhibition of the microorganism activity (Yong et al., 2014). In recent years, saline wastewater has been generated in many areas because seawater is used for toilet flushing to save freshwater resources. The used toilet flushing water is mixed with wastewater from other sources and the mixed wastewater is discharged into the sewage treatment plant. The salt concentration of the mixed wastewater is 5000 - 6000 mg/L, about one-fifth of the salt concentration in seawater (Wu et al., 2008).

1.6 CW-MFC microbiology

The microbiology of an integrated CW-MFC system is mostly governed by the EABs that impact wastewater treatment and bioelectricity production. It is well-reported that the abundance of the EABs is affected by their surrounding conditions and the symbiotic interaction with the electrodes as well as with the non-EAB microbial strains in their vicinity (Wang et al., 2019). The most dominant phyla associated with CW-MFC reactors are *Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes,* etc. These microbes are most abundantly reported in the literature to be associated with CW-MFC systems that are fed with organics (Gupta et al., 2021). At the genus level, the EAB population can also be affected by the anode pH. Wang et al. (2016), reported the abundance of *Geobacter* and *Dechloromonas* in neutral and slightly acidic pH, whereas the genera like *Fusibacter, Planctomyces, etc.* flourished in slightly alkaline environments. Another important aspect of CW-MFC microbiology is that, for an individual reactor the EAB abundance in the anode and cathode are dissimilar which is mostly due to different redox conditions. This signifies that anode and cathode biomass have different roles to play. For example, microbes like *Geobacter, Bacillus, Nitrospira,* etc are mostly reported to be abundant at the cathode region whereas *Firmicutes, Bacteroidetes, Commamonas,* etc. are associated with the anode (Gupta et al., 2021; Shahid et al., 2021).

1.7 CW-MFC State of the Art

Keeping in mind the potential of METs for simultaneous pollutant removal and energy production, the first study involving MET with CW by incorporating a microbial fuel cell in CW was performed by Yadav et al. (2012a), to obtain a high pollutant removal rate and reduce the land requirement. Since then the research interest on coupling of MET with CW which is known as iMETLand is growing constantly. Figure 1.9 represents the growing amount of interest and research patterns in iMETLand where wastewater treatment has been done by coupling an MFC with CW. The figure shows the increasing number of 4 publications in 2012 to 28 publications in 2019. The number of publications in 2020 is 11 to date and hopefully many are to come.

This technology has been under study for the last 10-12 years since its advent but it has not yet been established or developed as a suitable wastewater treatment scheme. Many of the past studies have concentrated on optimizing the power output maintaining the redox profile using synthetic and domestic wastewater and monitoring the COD (Cheng et al., 2006). The effectiveness of the system against high-strength industrial wastewater is still to be explored though. The performance of an MFC is dependent on many factors such as cathode and anode potential, substrate conversion rate, internal resistance of the reactor, and other factors related to the proton exchange membrane. However, the necessity of the proton exchange membrane

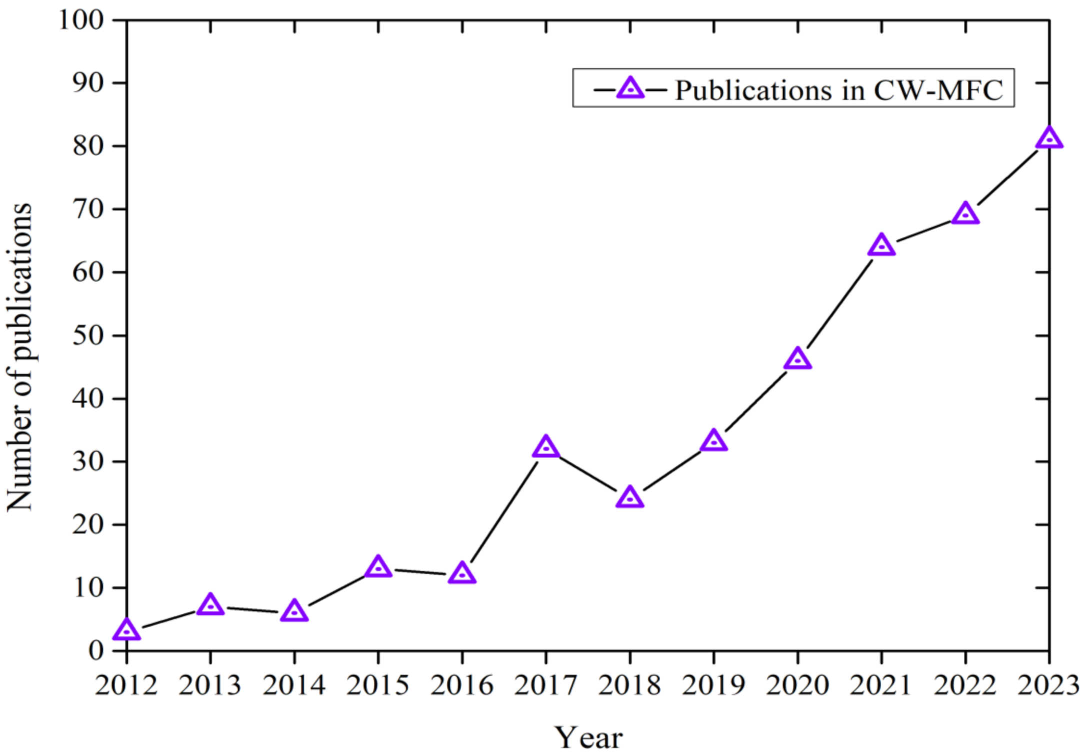

Fig. 1.9 Number of publications over the years in integrated CW-MFC systems

(Data was obtained from Scopus®. The search was performed using the terms Constructed Wetland and Microbial fuel cell (TITLE-ABS-KEY) and carefully monitoring the paper title & abstract in the results. For review & book chapters, only the articles focused on CW-MFC were considered after careful scrutiny.)

was found to be non-essential for up-flow reactors in many recent studies if sufficient distance is maintained between the electrodes (Sun et al., 2003). However, it may result in higher ohmic resistance. Some other influential factors that have been reported in the literature are pH, COD of the wastewater, effective surface area of electrodes, and electrode material (Logan, 2010). Doherty et al. (2015), operated CW-MFC using glass wool as the separation membrane in their experiment. The reactor flow regime was separated by the membrane. The flow was vertical upflow in the anode chamber and downflow in the cathode. This strategy was successful in terms of power generation but the effluent quality was poor due to a lack of available oxygen at the cathode vicinity.

A CW-MFC with a horizontal flow regime was operated by Villasenor et al. (2013), using bentonite as a separation layer between aerobic and anaerobic chambers. From the anode chamber, the flow was recirculated to the cathode. In this study, it was reported that higher organic loading reduced the power generated due to consumption of available oxygen in the cathode region thereby affecting the redox gradient. A comparative study on the performance of various CW-MFC systems has been compiled in Table 1.6. In most studies, graphite carbon or stainless steel mesh has been used as the system electrode. Many studies have used graphite or activated carbon in granular or gravel form to bury the anode to increase electricity production. The use of graphite, charcoal, or granular activated carbon can contribute to contaminant removal due to their adsorption property. It can be seen from Table 1.6, that the majority of the studies were performed with synthetic wastewater without focusing on the aspects of coliform inactivation from domestic wastewater. Another limitation was that none of these studies investigated the potential economic benefits of integrated CW-MFC reactors.

1.7.1 CW-MFC Cost Benefits

By combining an MFC with CW, cost benefits can be achieved. Integration of MFC with CW increases the rate of pollutant degradability which consequently reduces the reactor volume requirement thereby reducing the area footprint of the wetland itself. An additional advantage is the energy harvesting opportunity from the external circuit resulting from the metabolism of EABs. This integrated system has zero power consumption, low operation & maintenance cost, and facilitates the elimination of costly proton separation membranes. This system can also be operated with cheap and recyclable materials. Xu et al. (2018b), developed a cost-benefit model for MFCs attempting a comprehensive analysis of the prospective economic benefits. The model was applied to MFC, constructed wetland MFC, and MFCs with different anode and cathode materials. The study revealed that the constructed wetland MFC (CW-MFC), produced the highest revenue per unit of power production, and reflected the best cost-benefit ratio compared to the other systems. However, that study was mostly based on the experimental data that were obtained from the literature. It is expected that this cost-benefit model would not produce identical results when applied to MFC reactors in practical field-scale conditions treating raw wastewater and this warrants further investigation.

1.8 Knowledge gap and research challenges

The coupling of MFC with CW is an emerging technology that is at a preliminary stage and there is plenty to design, modify, explore, and optimize this novel integrated system. Though researchers have initiated several attempts to use this technology against diverse wastewaters with many short-term studies, any long-term effort to develop and establish this CW-MFC as a suitable fully-fledged decentralized domestic wastewater treatment scheme has not yet been made, especially in India. In most of the studies regarding domestic wastewater treatment with CWs, the testing of effluents for fecal coliform is less investigated or the results achieved are

Table 1.6 Summary of selected studies on CW-MFC

Wastewater characteristics	Study objective	Reactor Set up	Electrode material and details	Maximum removal (%)	Limitation	Maximum energy output	Reference
Synthetic wastewater COD was varied from 400 to 1000 ppm. pH = 7.2±0.2	Reduction of methane (CH_4) emission from CW in which CH_4 is controlled by MFC	Volume: 9.85 L Plant: *Typha orientalis* *Thalia dealbata* *Cyperus alteniforius* Continuous upflow HRT: 1 to 3 days	Stainless steel mesh was used as both anode and cathode. Granular activated carbon was used to form cathode (10cm) and anode (5cm) layer.	COD: 84.7 CH_4 emission reduction was 45%.	Economic benefits not investigated. Coliform removal was not monitored.	PD: 0.27 W/m^3 CD: 0.102 A/m$_3$ CE: N.A.	(Zhang et al., 2020)
Synthetic wastewater NH_4-N was 40 ppm and COD 800 ppm	Wastewater treatment	Three identical reactors. Horizontal and followed by vertical. Horizontal Volume: 6.4 L Vertical reactor volume: 27 L Plant: common sedges HRT: 15 hours	Graphite plate was used as both anode and cathode buried into graphite gravel layer.	TN: 90±1.15 NH_4-N: 94.4±0.75 TOC: 64.8±3.0 COD: 99.5±3.4	Economic benefits not investigated. Coliform removal was not monitored. Multiple reactor unites were used which gives higher area footprint.	PD: 224 mW/m^3 CD: 1706 mA/m^3 CE: 6.67%	(Srivastava et al., 2020b)
Synthetic wastewater C/N ratio: 200/40 in stage I and 800/40 in stage II	Nitrogen and Phosphorus removal with simultaneous bio-energy harvesting	Volume: 3.75 L Flow regime: downflow Plant: *Canna indica* HRT: 6 hours	Carbon fiber felts were used as both anode and cathode	COD: 71.9 NO_3-N: 70.1 TP: 91.2	Cost benefit analysis was not performed. Pathogen removal was not reported.	PD: 2.67 mW/m^2 in stage I and 6.74 mW/m^2 in stage II. CD: 24.77 mA/m^2 in stage I and 47.77 mA/m^2 in stage II.	(Ge et al., 2020)

Table 1.6 continued

Synthetic wastewater with COD around 1058 ppm	wastewater treatment and power generation	Volume: 30 L Flow regime: upflow HRT: 1.5 days Plants: N.A.	Carbon fiber felt and stainless steel mesh were used as both electrodes	COD: 91.7 NH_4-N: 97.3	Coliform removal is not checked.	PD: 7.99 mW/m^2 CD: N.A. CE: 0.36%	(Tang et al., 2019)
Synthetic wastewater COD: 210.4 ppm NH_4-N: 19.7ppm NO_3-N: 4.78ppm	To determine the role of plants in municipal wastewater treatment and bio-energy production.	Volume: 5.65 L HRT: 5 to 11 days Flow regime: upflow Plants: *Iris pseudacorus, Phragmites australis, Hyacinth pink*	Stainless steel mess filled with graphite gravel was used as both anode and cathode	COD: 51.6 NO3-N: 97.4 NH4-N: 71.5 Phosphate: 97.6	Economic benefits not investigated. Coliform removal was not monitored. High HRT was required.	PD: 25.14 mW/m^2 CD: 86.68 mA/m^2 CE: 9.74%	(Yang et al., 2019 b)
Synthetic wastewater	To determine the significance of water level in cathode potential.	Volume: 5.65 L HRT: 3 to 15 days Flow regime: upflow Plants: N.A.	Stainless steel mesh buried into graphite gravel was used as both anode and cathode.	N.A.	Economic cost-benefit anlysis was not performed. High HRT was adopted.	PD:165 mW/m^2 Voltage: 887.6 mV CE: N.A.	(Yang et al., 2019 a)
Synthetic wastewater COD: 300 ppm Reactive dye: 425 ppm	Decolorization of the azo dye-containing wastewater.	Volume 10.3 L HRT: 1.5 days Flow regime: upflow Plants: *Ipomoea aquatica*	Stainless steel mesh buried into granular activated carbon was used as both anode and cathode.	Decolourization amount: 130 ppm	Coliform removal was not investigated.	CD: 0.539 A/m^2 Voltage: 539 mV CE: 0.86%	(Fang et al., 2018)
Urban wastewater	Wastewater treatment.	Volume: 48L Flow regime: Horizontal HRT: 2 to 4 days	Stainless steel mesh was used as both anode and cathode.	COD: 74 NH_4-N: 41	Pathogen removal was not investigated.	CD: 50±7 mA/m^2 PD: N.A. CE: N.A.	(Hartl et al., 2019)

Table 1.6 continued

Synthetic wastewater simulating medium to high-strength sewage water	Wastewater treatment along with electricity generation.	Volume:143.2 L Flow regime: horizontal with effluent recirculation HRT: 6.6 days Plants: *Canna indica*	Graphite rods and graphite gravels were used as both anode and cathode.	COD:98.9±2.34	High HRT was employed. Cost-benefit aspects was not discussed.	PD: 11.67 mW/m^3 CD: 17.15 mA/m^3 CE around 2%	(Srivastava et al., 2020a)
Synthetic wastewater Glucose: 200 mg/L NH_4Cl: 150 mg/L KCl: 130 mg/L	To determine the role of wetland vegetation on CW-MFC performance.	Volume: 81 L Flow regime: upflow HRT: 4 days Plants: *Typha latifolia, Typha angustifoolia, J.gerardii, C.divisa*	Graphite plate was used as the anode and magnesium plate was used as the cathode.	COD: 88 NH_4-N: 98 NO_3-N: 63 TP: 97%	The HRT was comparatively higher. Coliform removal was not focused.	CD: 33.8 mA/m^2 CE: 8.28±10.4% PD: 18.1 mW/m^2	(Saz et al., 2018)
Synthetic wastewater Nitrobenzene: 5-80 mg/L Glucose: 100-600 mg/L	Nitrobenzene degradation from wastewater	Volume: 38.85 L Flow regime: upflow HRT: 24 hours Plants: N.A.	Graphite bar was used as both cathode and anode.	COD: 78 Nitrobenzene: 92	Economic benefit analysis not reported.	CE: 16.4% PD: 1.53 mW/m^2 CD: 8.52 mA/m^2	(Xie et al., 2018)
Synthetic wastewater Sucrose: 53.48 mg/L, KNO_3: 50.5 mg/L, KH_2PO_4: 6.58 mg/L, $(NH_4)_2SO_4$: 37 mg/L	Generation of bioelectricity and microbial community development	Volume: 17.27 L Flow regime: upflow HRT: 3 days Plants: *Phragmytes australis*	Titanium mesh with activated carbon was used as the anode titanium mesh was used as the cathode	COD: 82 TN: 82 TP: 95	Coliform removal was not investigated.	CD: 16.63 mA/m^2 CE: N.A. PD: 3714 mW/m^2	(Xu et al., 2018a)

Table 1.6 continued

Synthetic wastewater Sodium acetate: 642 mg/L, NH_4Cl: 150 mg/L, $CaCl_2$: 16 mg/L, $MgSO_4$: 12 mg/L, and KH_2PO_4: 14 mg/L.	Influence of glass wool as a separator on bioelectricity production	Volume: 4.71 L Flow regime: upflow HRT: 24 hours Plants: N.A.	Carbon fiber felts were used as both cathode and anode.	N.A.	No cost-benefit analysis was performed. Coliform removal not reported.	PD: 26.12 mW/m^2 CD: N.A. CE: N.A.	(Xu et al., 2018c)
Synthetic wastewater Glucose: 200 mg/L, NH_4Cl: 150 mg/L, KCl: 130 mg/L, $NaHCO_3$: 3.13 g/L	Bioelectricity production in CW-MFC and effect of substrate on wastewater treatment.	Volume: 46L Flow regime: upflow HRT: 4 days Plants: *Typha latifolia*	Graphite plate was used as the anode and magnesium plate was used as the cathode.	COD: 92 NH_4-N: 93 NO_3: 81 TP: 96	HRT adopted was comparatively high. Coliform removal was not focused.	PD: 26.12 mW/m^2 CD: 16.1 mA/m^2 CE: 1.64 %	(Yakar et al., 2018)
Synthetic wastewater H_3BO_3: 2-32 mg/L	Simultaneous Boron removal and bioelectricity production.	Volume: 81 L Flow regime: horizontal pulse flow HRT: 7 days Plants: *Typha latifolia*	Graphite rod was used as anode and magnesium bar was used as cathode	Boron: 63 NO_3: 47 NO_2: 19	The HRT adopted was comparatively higher.	CE: N.A. CD: 105 mA/m^2 PD: 78 mW/m^2	(Türker and Yakar 2017)
Synthetic wastewater Glucose: 0-2 mM	Methane emission control and bioenergy production	Volume: 2.55 L Flow regime: upflow HRT:1 to 4 days Plants: *S. alterniflora*	Granular activated carbon was used as both cathode and anode.	98% reduction of methanogenesis	Coliform removal was not reported.	PD: 76.7 mW/m2 CD: 187 mA/m2 CE: 14.9%	(Liu et al., 2017)

N.A.: Not available, CE: coulombic efficiency, CD: current density, PD: power density, HRT: hydraulic retention time, TN: total nitrogen, TP: total phosphorus not appreciable. To achieve a higher level of coliform removal, elongated HRT or application of a hybrid wetland system with multiple reactor assemblies is required which surges the areal footprint of the constructed wetland. Also, there are acute limitations of studies that have focused on investigating and understanding the mechanism of fecal microbe inactivation in integrated CW-MFC reactors.

The presence of pathogenic microbes in the effluent of conventional wastewater treatment plants is a major hurdle for wastewater reuse in domestic and irrigation sectors. Municipal wastewater contains a substantial amount of fecal pathogens. Conventional wastewater treatment schemes like ASP or trickling filters are not designed for their removal thus the presence of pathogenic microbes in the effluent of conventional wastewater treatment units becomes a major hurdle for wastewater reuse in domestic and irrigation sectors. However, the number of studies emphasizing the removal of fecal coliforms from domestic wastewater is very limited in the literature. Many constraints are still associated with CW-MFC regarding the understanding of the design, selection of electrode materials, optimizing the specific electrode area to reactor volume ratio, best cathode position; electrode spacing, and specific substrate condition in the electrode region and separation zone. These need to be studied further for scaling up CW-MFC as the amount of literature demonstrating their specific roles and effects on the system is less. The microbial community associated with a CW-MFC system can be broadly classified into (i) electroactive bacteria (EAB) (ii) methanogenic bacteria (iii) heterotrophic bacteria, and (iv) nitrifying bacteria. The interrelation, relative dominance, and competition among these three types of microorganisms should be thoroughly studied and explored. It is also important to investigate if the presence of electrodes as external electron acceptors is capable of inducing subtle or significant changes to the reactor biomass population as compared to standalone CW reactors.

In long-term CW-MFC reactor operations, there is a possibility of exerting a negative impact on the anodic anaerobic activities from the macrophyte roots via radial oxygen loss. Lab-scale reactors are more vulnerable to this due to small reactor size. When operated for more than a year, certain probabilities of plant roots growing and reaching the anode vicinity may hamper anaerobic EAB activity. Unfortunately, the number of studies focusing on this aspect is limited and this needs to be further investigated for extended reactor operation.

The proposal or plan for establishing any decentralized treatment scheme should be based on a thorough analysis of prospective economic benefits in terms of effluent reuse and wastewater valorization. This could be achieved through an exhaustive and cost-benefit analysis including the investment cost, operation and maintenance cost, and probable benefits from wastewater treatment and reuse. Though CW-MFC has the additional advantage of bioenergy production with wastewater treatment, very few articles have focused on probing this area. Cost-benefit analysis of integrated CW-MFC systems treating wastewater generated from diverse sources and operating under different retention times and environmental conditions could be a key factor for establishing this technology in rural or suburban sectors.

In recent years, many environmental scientists have focused on the application of multi-anode CW-MFC reactors for the enhancement of the bioelectrical performance of the reactors and also to generate high-quality effluent (Xie et al., 2024; Tamta et al., 2023). However, very few articles have investigated the prospect of multi-anode CW-MFC reactors for the inactivation of pathogenic microbes from domestic wastewater.

1.9 Research protocol and objectives

The main focus of this research was to investigate the raw domestic wastewater treatment performance of an integrated CW-MFC reactor for simultaneous organics and fecal coliform removal and bioelectricity generation to establish it as an effective decentralized wastewater treatment technology. The performance of the integrated CW-MFC reactor was compared with a standalone CW reactor and an unplanted CW-MFC reactor. The associated bioelectrochemical mechanisms were investigated along with monitoring the role of seasonal effects and reactor operational parameters for long-term reactor operation. Additionally, a cost-benefit model was employed to assess and obtain the cost-benefit ratio of the three reactors for an operational period of 600 days. Figure 1.10 elucidates the research protocol flowchart used in the current study.

The sequestration of the research for achieving the objectives is enlisted below:

- Treatment of low-strength domestic wastewater focusing on organics removal and fecal coliform inactivation with the assessment of seasonal performance variation and bioelectricity production.
- Assessment of the biomass population of the reactors to see the difference in the diversity and dominance of EABs in integrated CW-MFC reactor (R2) as compared to the CW reactor (R1) and unplanted CW-MFC reactor (R3).
- Evaluation of bioelectrochemical mechanisms involving organics and fecal coliform inactivation in integrated CW-MFC system.

- Investigation of the roles of media and macrophyte in long-term reactor operation under varying operational conditions and its effects on bioenergy production.
- Comparative cost-benefit analysis of three different reactors to identify the best option for sustainable and economic domestic wastewater management.
- Application of multi-anode CW-MFC reactor for enhancing the bioelectricity generation and wastewater treatment performance as compared to single-anode reactor.

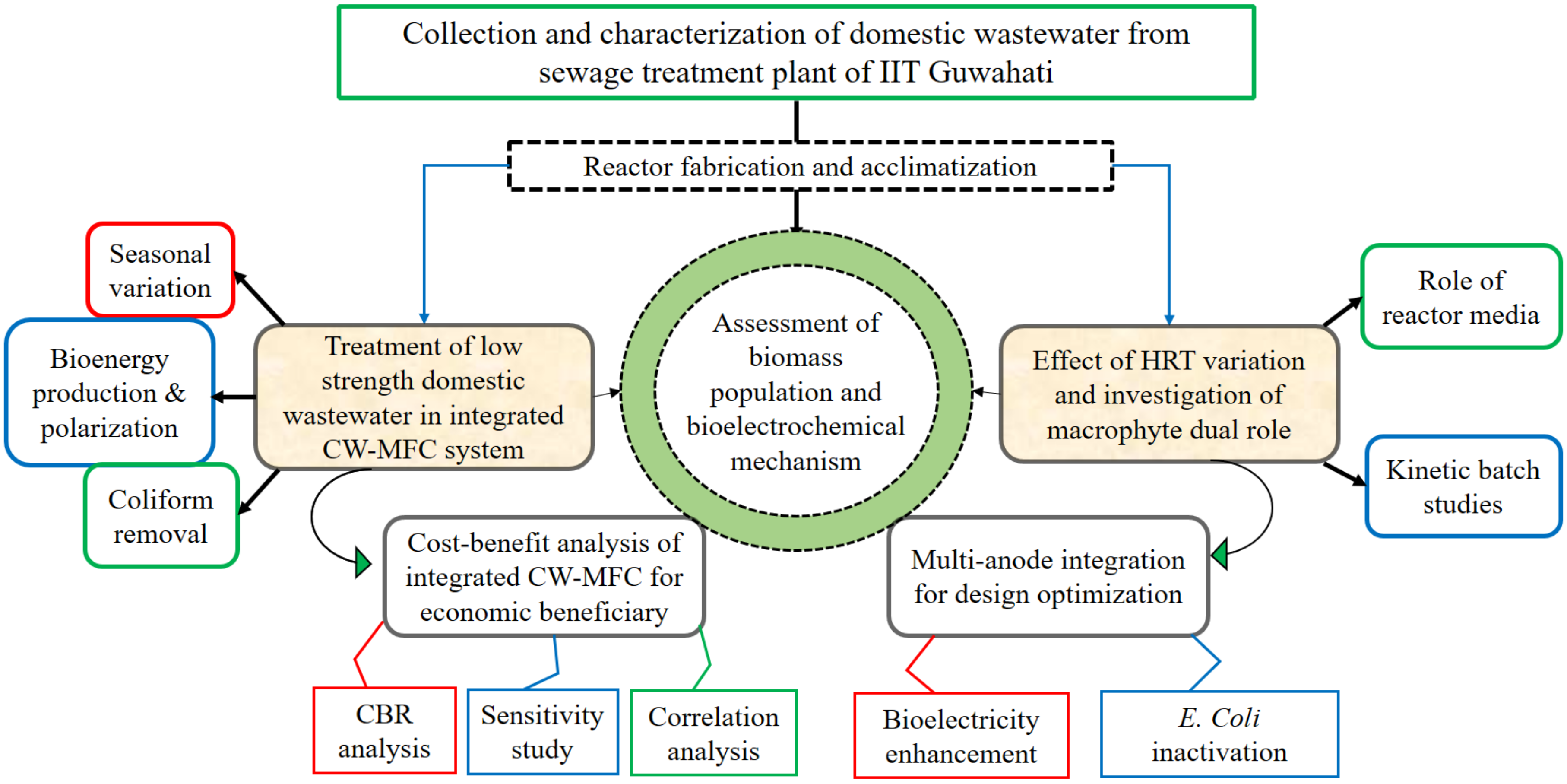

Fig. 1.10 Flowchart of the research protocol used in the current study

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Chapter 2

Treatment of Low-Strength Domestic Wastewater in Integrated CW-MFC Reactor with Coliform Inactivation and Bioenergy Yield

This chapter encapsulates the fabrication, operation, and monitoring of three lab-scale vertical reactors for the treatment of low-strength domestic wastewater. One integrated constructed wetland microbial fuel cell (CW-MFC), one constructed wetland (CW), and one unplanted CW-MFC were used for the assessment of organics removal, coliform inactivation, and bioenergy harvesting.

2.1 Introduction

Multiple researchers have documented synthetic domestic wastewater treatment using CW-MFC (Oon et al., 2016; Wang et al., 2016), any long-term effort to treat domestic wastewater considering the fluctuations of wastewater characteristics and the assessment of seasonal performance variation is rare. Most of the CW-MFC studies have addressed organic and nutrient removal and are very less focused on pathogen inactivation which is a major source of contamination in low strength domestic wastewater. In most of the studies regarding coliform removal with CWs, results achieved for fecal coliform removal are either not appreciable (1 – 2 log removal) or it is cost-intensive (Chand et al., 2021; Lekhsmi et al., Calheiros et al., 2021; Vymazal, 2005).

The objective of the current chapter is to evaluate the long-term performance of the planted CW-MFC system treating original low-strength domestic wastewater for the removal of organics and pathogens by monitoring the effects of seasonal variation and to compare the coliform removal and organic removal performances with planted CW and unplanted CW-MFC reactors having identical configuration, wetland media, hydraulic loading, and organic loading rates. Another objective was to study and compare the energy harvesting from planted and unplanted CW-MFC reactors with an assessment of the contribution of the plants to enhance bioelectricity generation.

2.2 Materials and methods

2.2.1 Reactor fabrication and media

Three identical reactors were fabricated using PVC pipes named R1 (planted CW), R2 (planted CW-MFC), and R3 (unplanted CW-MFC). These reactors were installed in an open environment at the Department of Civil Engineering, Indian Institute of Technology Guwahati, Assam, India. The shape of the reactors was cylindrical, with a diameter of 20 cm and a height of 40 cm. Three sampling ports (S1, S2, and S3) were made at a distance of 12 cm (anode), 20 cm, and 30 cm (cathode) respectively from the bottom inlet point. These ports were used for sampling and analysis of the reactor effluents. Stainless steel mesh (SSM), graphite rods (10 mm dia, 300 mm long), and granular activated carbon (GAC) (5-10 mm) were used as the electrode materials for the R1 and R3 reactors. The anode and cathode were externally connected to a resistor with copper wire. Gravels (20 mm passing and 10 mm retaining) were used in all the reactors as the drainage layer and the separation layer between the anode and the cathode zone. A mixture of locally available soil and gravel was used as the top layer in all three reactors. Peristaltic pumps (BT50S) were used for reactor operation in a continuous feeding mode at a constant rate.

2.2.2 Experimental setup and reactor operation

The effective volume of the reactors was 4.5 – 4.6 L, which was calculated by measuring the required volume of tap water to fill the compacted reactors, after media compaction and plantation. Gravel was used as the filler media in reactors R1, R2, and R3. Stainless steel mesh (SSM) and graphite rods were used as anodes and graphite rods were used as cathodes in R2 and R3 reactors. The electrodes were buried into a 2 cm thick GAC layer and the graphite rods were extended out of the reactors and connected with an external resistor by copper wire. GAC is extensively used in literature as electrode layers as a low-cost bio-compatible electrode material for increasing electrical performances (Sun et al., 2011). Considering the adsorption capabilities of activated carbon, the GAC layer thickness was kept negligible (2 cm) compared to the overall reactor height (40 cm). Reactor R1 was also provided with a GAC layer to negate the pollutant removal contribution of GAC while performance comparison but the SSM and the graphite rods with external circuit setup were omitted. The anode was placed 10 cm above the bottom surface. The cathode was placed 28 cm above the bottom surface providing a separation layer of 16 cm between the electrodes. Effluents were collected at the sampling port

(S3) from all the reactors to analyze the organics, pH, total coliforms, and fecal coliform concentrations. Samples collected from S1, S2, and S3 were analyzed for redox potential (ORP), and samples collected from S3 were analyzed for dissolved oxygen (DO) in all the reactors. Figure 2.1 illustrates the schematics of the reactor system.

The reactors were operated in continuous mode with a hydraulic retention time (HRT) of 36 h. HRT was fixed by maintaining 125 mL/h flow rate of the peristaltic pumps. This corresponded to a discharge volume equal to the effective volume of the reactors within the stipulated period of 36 h. During the acclimation period (first 15 days of operation), raw wastewater was diluted up to 50% by adding tap water before feeding the reactors. After the acclimation period was over, raw sewage was directly fed into the reactors and the study was continued for 325 days. During the operation, the hydraulic loading rate (HLR) was maintained at 95.54 L/m^2.d.

2.2.3 Domestic wastewater and wetland macrophyte

The domestic wastewater was collected at regular intervals, from the grit chamber of the sewage treatment plant (STP) of the Indian Institute of Technology Guwahati (26°11'N 91°42'E) and fed to the reactors using peristaltic pumps. The wastewater was also characterized in the laboratory by analyzing the 5-day biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), pH, ammonia (NH_3-N), total suspended solids (TSS), total dissolved solids (TDS), total coliforms (TC), and fecal coliform (FC). The result of wastewater characterization during the study is summarized in Table 2.1. During the study, the highest and the least concentration of COD of the wastewater was observed to be 212.55 mg/L and 140.0 mg/L, leading to a maximum and minimum organic loading rate (OLR) of 20.30 g/m^{2}d and 13.37 g/m^{2}d. Locally available macrophyte, *Typha angustifolia* (narrow-leaved cattail) was collected from the wetland of the IIT Guwahati campus and was planted in R1 and R2 reactors. Two plants were placed in each reactor resulting in a stem density of 70 plants per m^2 area. At the time of collection, the average plant height was 45.78 cm. *Typha angustifolia* was selected as the macrophyte due to its easy availability inside the campus area and its high tolerance to diverse wastewater (Jinadasa et al., 2008; Yadav et al., 2012).

2.2.4 Analytical methods

During the study, effluents from all the reactors were collected periodically and were analyzed for COD, BOD, pH, total and fecal coliforms. The pH was measured with a digital pH meter.

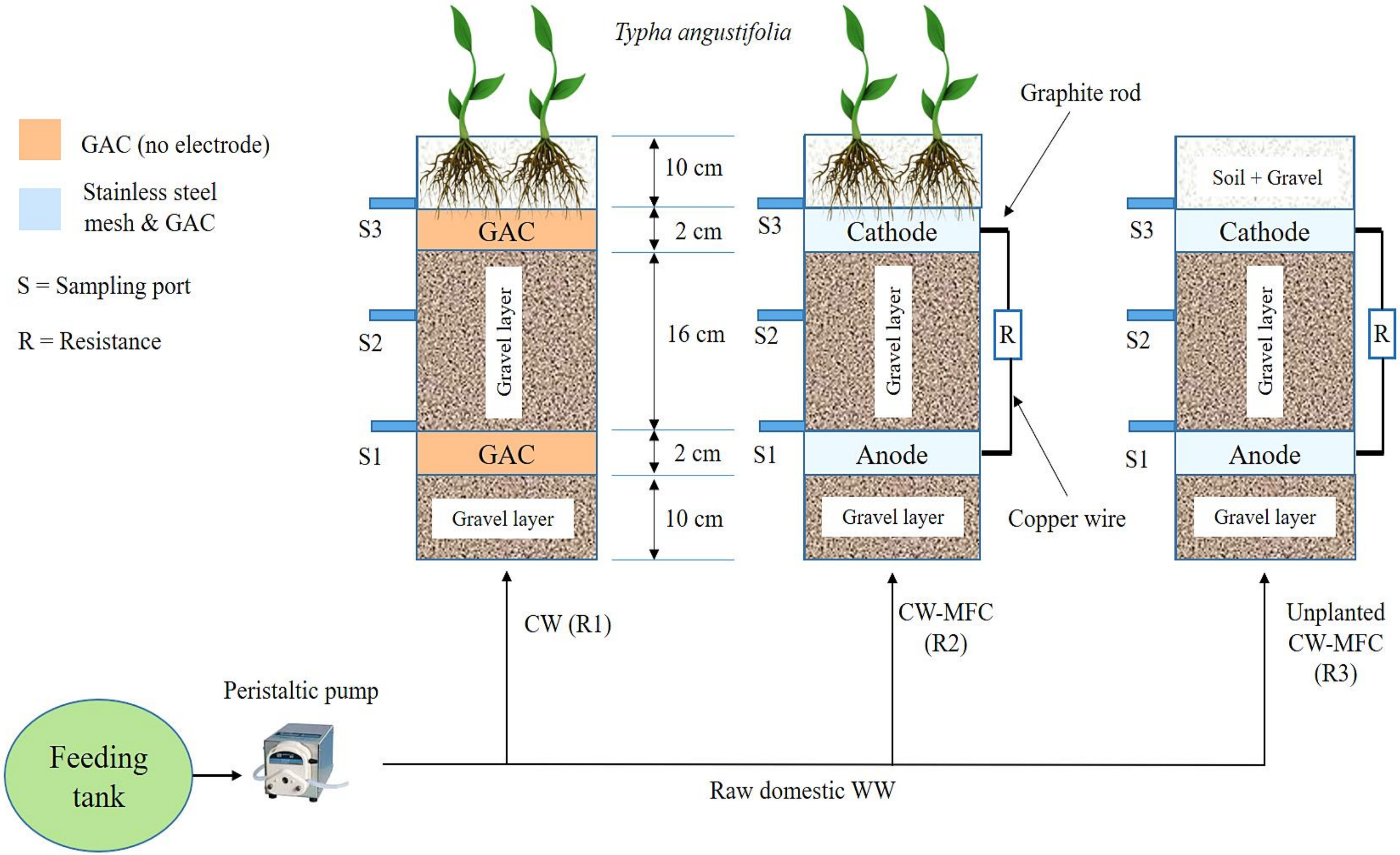

Fig. 2.1 Schematic diagram of the reactor system

Table 2.1 Characterization of raw domestic wastewater

Parameter	Concentration	Instrument/methods used
COD	169.89±15.37 mg/L	HACH COD digester/Closed reflux method
BOD_5	99.65±9.19 mg/L	BOD incubator (HTLP-020)
pH	7.16±0.09	Systronics µ 361
Ammonia	20.05±1.33 mg/L	Visible spectrophotometer (mrc V11D)/Phenate method
TSS	167.75±16.65 mg/L	N.A./Gravimetric method
TDS	277.85±21.22 mg/L	N.A./Gravimetric method
Total coliform	(12.06±5.78)E+06	BOD incubator (HTLP-020)/Multiple tube fermentation
Fecal coliform	(8.87±5.86)E+06	BOD incubator (HTLP-020)/ Multiple tube fermentation

N.A.: Not applicable

The open circuit voltage (OCV) and operating voltage (OV) in the R2 and R3 reactors were measured using a portable digital multimeter (MAS/830L). Redox potential was measured using a digital ORP meter (HANNA, HI 98201). Measurement of DO and ORP was done on the spot immediately after sample collection. Chemical oxygen demand (COD), 5-day biochemical oxygen demand (BOD_5), and total and fecal coliform analyses were performed as per the procedure given in the standard methods (APHA, 2017). DO of smaller volumes, collected from sampling port S3 of the reactors was measured according to Shriwastav et al. (2010).

The HRT, organic loading rate (OLR), reactor surface area, and removal efficiency were calculated using equations 2.1, 2.2, 2.3, and 2.4 respectively.

$$HRT\ (h) = \frac{Effective\ reactor\ volume\ (m^3)}{Wastewater\ flow\ rate\ \left(\frac{m^3}{h}\right)} \tag{2.1}$$

$$OLR\ \left(\frac{g}{m^2}/d\right) = \frac{Influent\ organic\ concentration\ \left(\frac{g}{L}\right) \times flow\ rate\ \left(\frac{L}{d}\right)}{Surface\ area\ of\ the\ reactor\ (m^2)} \tag{2.2}$$

$$Reactor\ surface\ area\ (m^2) = \pi\frac{d^2}{4} \quad (d\ is\ reactor\ diameter\ in\ m) \tag{2.3}$$

$$HLR\ \left(\frac{L}{m^2}/d\right) = \frac{flow\ rate\ \left(\frac{L}{d}\right)}{Surface\ area\ of\ the\ reactor\ (m^2)} \tag{2.4}$$

$$Organic\ removal\ efficiency\ (\%) = \frac{S_o - S_e}{S_o} \times 100 \tag{2.5}$$

Where, S_o= influent concentration, S_e = effluent concentration.

$$Log\ removal\ for\ coliforms = log\ \frac{N_o}{N} \tag{2.6}$$

Where, N_o = MPN/100 mL in raw wastewater, N = MPN/100 mL in reactor effluents.

The first-order removal rate coefficient of 36 h for fecal and total coliform was determined by the equation given below (Sperling and Paoli, 2013),

$$N = N_o\ (e^{-KT}) \tag{2.7}$$

Where, N = MPN/100 mL in reactor effluents, N_o = MPN/100 mL in raw wastewater, T = HRT of the reactors (36 hours), and K = First order removal rate coefficient for 36 h retention time (K_{36}).

Current (I), Power (P), Power density (PD), current density (CD), and coulombic efficiency (CE) were calculated using equations (2.7), (2.8), (2.9), (2.10), and (2.11).

$$I\ (A) = \frac{V\ (volt)}{R\ (ohm)} \tag{2.8}$$

$$P\ (W) = V\ .\ I \qquad (V\ is\ voltage\ and\ R\ is\ resistance) \tag{2.9}$$

$$PD\ \left(\frac{W}{m^2}\right) = \frac{P\ (W)}{Area\ of\ anode\ electrode\ (m^2)} \tag{2.10}$$

$$CD\ \left(\frac{A}{m^2}\right) = \frac{I\ (A)}{Area\ of\ anode\ electrode\ (m^2)} \tag{2.11}$$

$$CE = \frac{M.\ I}{b.\ F.\ \triangle COD.\ q} \tag{2.12}$$

Where, M, b, F, and q are the molecular mass of O_2 (32 g/mol), the number of electrons donated by 1 mol O_2 (4), Faraday's constant which is 94,685 (Coulomb/mol), and flow rate (L/s). △COD is the difference in influent and effluent COD concentration (mg/L).

2.3 Results and discussion

2.3.1 Redox potential and dissolved oxygen

As illustrated in Figure 2.2 (a), ORP was low at sampling ports S1 and S2 in all the reactors, and the highest ORP was observed at sampling port S3, which is indicative of an organic content reduction in the wastewater throughout the reactor length (Zhai et al., 2012). However, ORP at S3 in reactor R3 (unplanted) was found to be comparatively lower compared to that of the planted reactors i.e. R1 and R2, which suggests a positive role of plants in increasing the redox potential. To investigate further, DO was measured at sampling port S3 in all the reactors. In reactor R3, the observed DO at S3 was 0.22±0.08 mg/L. In the planted reactors (R1 and R2) the observed DO was comparatively higher. The mean DO levels of reactors R1 and R2 were found to be 2.19±0.06 mg/L and 1.97± 0.07mg/L. The presence of higher DO near the root zone of the planted reactors may be attributed to the release of oxygen by the *typha* roots (Wießner and Kuschk, 2002; Di et al., 2020). Camacho et al. (2014), have reported redox potential within 30-35 mV while treating synthetic domestic wastewater in vertical flow CW planted with *Phragmites australis* with an effluent DO of 3-4 mg/L. In another study, Hussein and Scholz (2017), reported ORP between -32 mV to 6 mV in a vertical flow CW planted with *Phragmites australis* treating synthetic dye wastewater. They have reported DO values between 5.4 to 8.2 mg/L in the effluent. However, there is always a high possibility of entrapping air from the atmosphere while measuring DO which may lead to higher concentration.

2.3.2 Variation of pH in wastewater and reactor effluents

Figure 2.2 (b) illustrates the pH variation of R1, R2, and R3 reactors along with the pH of raw wastewater. In the current study, the average pH in the effluents of R1, R2, and R3 is shown in Table 2.2. The near-neutral pH range of all three reactors' effluents supports high levels of organic removal. In the integrated system, during the carbon and nitrogen removal processes, proton (H^+) production at the anode and proton consumption at the cathode leads to a potential pH drift which adversely affects the function of the biofilm (Sarkar and Jones, 1982). The pH can also be a factor for plant selection in CW-MFC and wetland systems. MFCs have generally shown better performance around neutral pH, which is indicative of applying macrophytes to CW-MFC systems which thrive best at the neutral pH range. This can lead to increased redox potential and higher voltage and current production (Blossfeld et

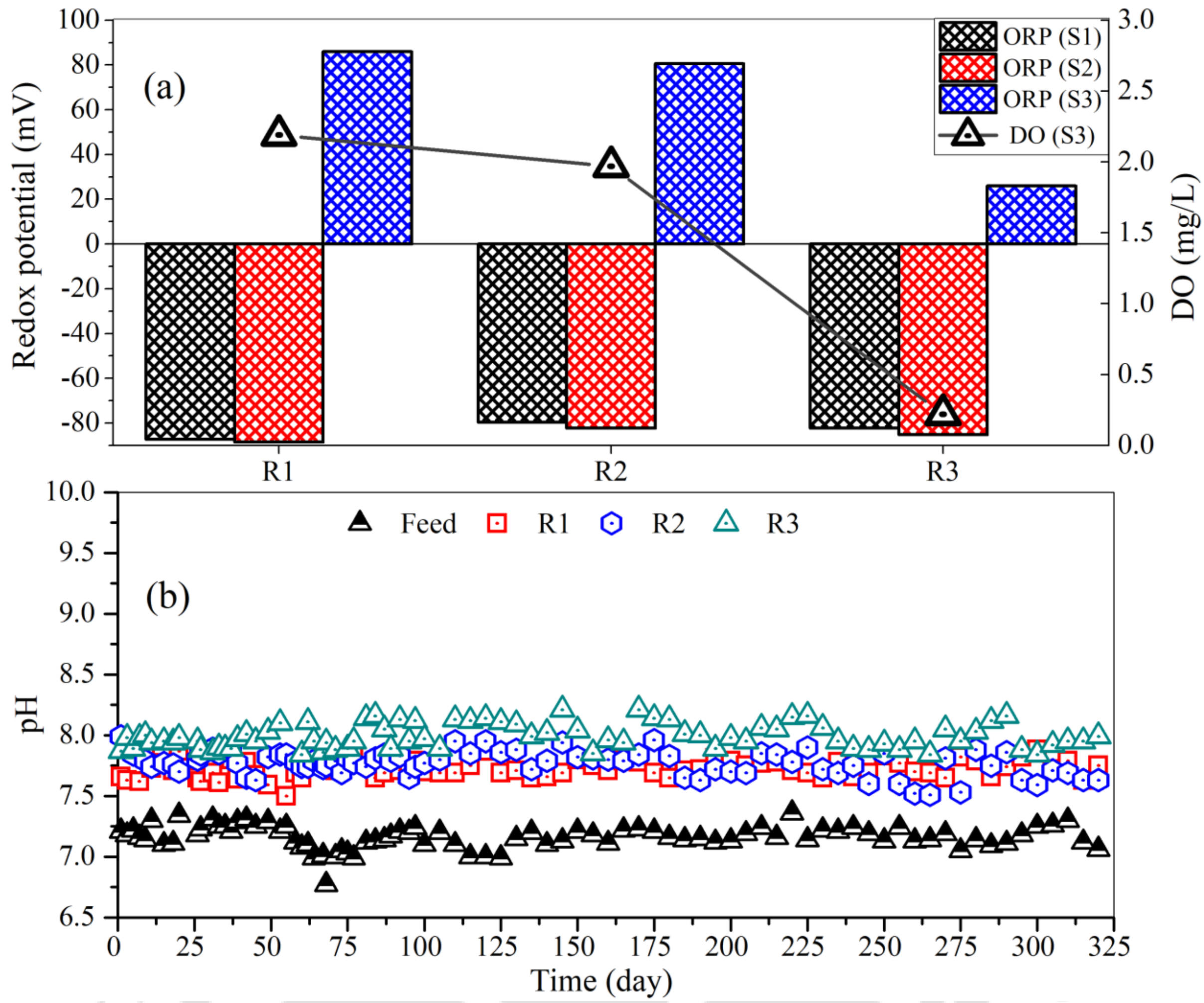

Fig. 2.2 Variation of (a) ORP and DO of the reactor effluents obtained from different sampling ports and (b) pH in raw wastewater and reactor effluents during the study

al., 2010). While operating CW-MFC, a slight pH variation is expected due to microbial activity but a high pH gradient within the electrodes leads to a deterioration in the system performance (Regmi et al., 2018). Considering the effluent characteristics in the current study, the pH drift was higher in reactor R3 than in reactor R2, which also justifies the better performance of the integrated reactor.

2.3.3 Organic removal from domestic wastewater

The COD concentration of the feed and the reactor effluents throughout the study period is illustrated in Figure 2.3 (a). The integrated CW-MFC reactor (R2) performed better than the standalone CW (R1) and unplanted CW-MFC (R3). The mean COD of the feed wastewater was 169.89±15.37 mg/L. The mean organic loading rate (OLR) and hydraulic loading rate (HLR) for all three reactors were 16.23 g/m^{2}d and 95.54 L/m^{2}d, respectively.

Reactor R2 showed the highest COD removal. It was comprised of plants and electrodes with an external circuit for the transfer of electrons. Organics present in wastewater have played their role as electron donors during degradation and electrons are transferred from the anode to the cathode at the top via the external circuit. R2 showed an ORP value of +80 mV near the cathode, suggesting an aerobic environment. *Typha angustifolia* present in R2 was responsible for higher DO concentration near the cathode due to root level release of oxygen. Oxygen in the cathode worked as an electron acceptor. So, the external circuit helped in electron transfer and plants played their role to supply oxygen as the terminal electron acceptor. In reactor R1, ORP was similar to R3 at the top as plants were present, suggesting the availability of oxygen near the root zone. However, electron transfer was limited due to the absence of the external circuit so COD removal was lower than R2. In reactor R3, the electrodes were associated with an external circuit, which helped the electron transfer (donated by wastewater organics) from anode to cathode but the ORP value was low near the cathode (25 mV), suggesting less availability of oxygen as terminal electron acceptor. This study showed that both electron transfer from the anode and the presence of electron acceptors at the cathode increased organics removal. The increased COD removal in R2 in the current study can be correlated to the findings of Xu et al. (2018), and Srivastava et al. (2015). These studies showed that CW-MFC performed comparatively better than CW for the treatment of synthetic and domestic wastewater.

Ramírez-Vargas et al. (2019), obtained 90% COD removal using a CW-MFC system with a minimum OLR of 8.9 g/m^{2}d and influent COD of 381 – 410 mg/L. Fang et al. (2015, 2013), and Yadav et al. (2012), obtained 85%, 85.7%, and 75% COD removal efficiency with an influent COD of 180 – 205 mg/L in synthetic wastewater. The performance of R1 in terms of COD reduction was comparable with the literature. Foladori et al. (2013), obtained 85% COD removal using a vertical flow CW planted with *Typha angustifolia* and operated with an HLR of 164 L/m^2.d and an average influent COD of 233±76 mg/L. Wu et al. (2011), obtained a COD removal efficiency of 84±10% with an influent COD concentration of 193±44 mg/L. Ayaz et al. (2015), used a vertical subsurface flow CW planted with *Phragmites australis* for anaerobically pre-treated domestic wastewater treatment with influent COD of 566±140 mg/L and obtained a maximum removal efficiency of 92±3%. Pinninti et al. (2022), obtained a maximum COD removal efficiency of 91% with an HRT of 7 days while treating domestic wastewater in vertical flow CW planted with *Canna indica*. The COD removal performance of reactor R3 was somewhat lower than most found in the literature. Xie et al. (2011), reported a COD removal efficiency of 98% while treating synthetic wastewater. Similar efficiency was observed by Pandit et al. (2012), who reported 93% organic carbon removal.

Figure 2.3 (b) depicts the BOD_5 of the feed and different reactor effluents with time. Not much variation in performance was observed in terms of BOD_5 removal among reactors R1, R2, and R3. During the acclimation period, the effluent BOD_5 of reactor R2 was found in the range of 7 – 22 mg/L. After the acclimation, the average effluent BOD_5 was 7.25±3.55 mg/L. Reactors R1 and R3 rendered effluent BOD_5 within 9.5 – 22.0 mg/L and 14.1 – 22.0 mg/L during the acclimation. Post acclimation, the mean effluent BOD_5 levels for R1 and R3 were reduced to 8.2±3.88 mg/L and 7.85±3.86 mg/L. The average BOD_5 and COD removal efficiencies of R1, R2, and R3, during winter and summer are given in Table 2.2. During the summer, BOD_5 and COD removal performance of all the reactors was increased compared to winter which is possibly due to enhanced heterotrophic activity of the microbes in the raw domestic wastewater. During winter, the mean ambient temperature varied between 18.3 to 23.0 °C and in the summer it was 23.0 to 31.4 °C. The effluent water temperature from all the reactors was found to be higher than the ambient temperature. In winter the average water temperature varied between 20.3 to 26.5 °C and during summer it was found to be 26.5 to 35.5 °C.

Aguirre-Sierra et al. (2014), reported 93% BOD removal while treating domestic wastewater with integrated CW-MFC reactor. Similar results to the current study were reported by Ramírez-Vargas et al. (2019), where no significant difference in the planted and the non-planted electroactive wetlands were found. Both the reactors exerted around 85 – 90% BOD removal. The high BOD_5 removal performance in all the reactors may be attributed to the readily biodegradable nature of the raw wastewater which has a BOD_5/COD ratio of 0.58. As it may be observed, in terms of COD, the integrated system showed better performance, but no significant difference was found in BOD_5 removal from different reactors. The mean BOD_5/COD ratio in the effluents of R1, R2, and R3 were 0.37, 0.61, and 0.33 respectively. BOD_5 generally represents the amount of dissolved oxygen utilized by the microorganisms for oxidizing the biodegradable organics and COD results can include refractory organic compounds capable of reacting with dichromate, or other organics that may be toxic to the bacteria and, therefore might not be able to contribute to BOD_5. The presence of anode as the electron acceptor in the anaerobic environment tends to accelerate the organic

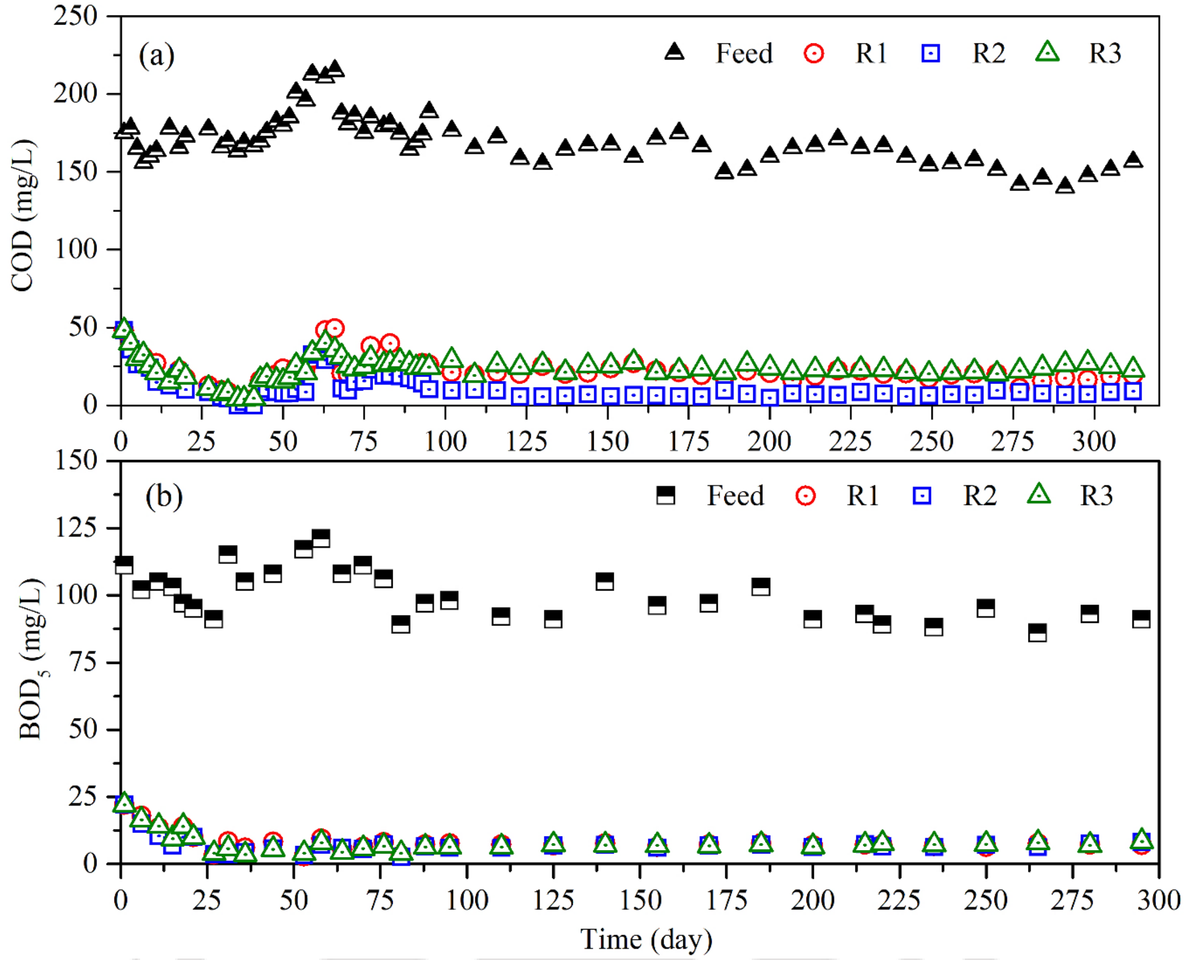

Fig. 2.3 Variation of COD and BOD_5 in the raw wastewater and reactor effluents in the study

Table 2.2 Summary of reactor performance during summer and winter

Parameters	Summer			Winter		
	R1	R2	R3	R1	R2	R3
COD removal efficiency (%)	86.44±7.56	94.36±8.52	85.26±8.41	85.60±6.54	91.75±7.31	84.96±8.05
BOD_5 removal efficiency (%)	93.51±8.67	92.93±7.22	93.32±6.51	88.44±7.22	89.66±7.11	88.35±8.31
Effluent pH	7.70±0.74	7.79±0.64	7.98±0.71	7.75±0.59	7.66±0.45	7.96±0.65
OCV (mV)	Nil	886±24.36	487±22.74	Nil	872±27.51	487±13.59
TC log removal	3.06±0.16	3.33±0.25	3.11±0.15	2.59±0.17	2.73±0.21	2.68±0.24
FC log removal	4.31±0.21	5.22±0.35	3.89±0.23	3.81±0.19	4.09±0.29	3.49±0.27

OCV: Open circuit voltage, TC: Total coliform, FC: Fecal coliform. Data format: XX±YY, where 'XX' is the mean, and 'YY' is the standard deviation matter degradation. This happens as the EABs (*Geobacter*, *Shewanella*, *etc.*) preferably use the anode rather than using nitrite, sulfur, thiosulfate, etc. as electron acceptors (Bücking et al., 2013; Zhang et al., 2015). This explains the better removal efficiency in reactor R2 compared to that of reactors R1 and R3 for COD but not for BOD_5. This phenomenon is reflected in the reduced BOD_5/COD ratio of reactors R2 and R3 compared to reactor R1.

2.3.4 Coliform removal from domestic wastewater

Table 2.3 shows the mean coliform levels in the raw wastewater and effluents of R1, R2, and R3 reactors in the current study. The average fecal coliform (FC) and total coliform (TC) removal were above 99% in R2 corresponding to log removals of 3.95±0.17 and 3.18±0.17 respectively. In R1, average FC and TC removal were found to be log 3.34±0.11 and log 3.03±0.16 units. In the case of reactor R3, the FC and TC removal efficiencies were observed to be log 3.45±0.12 and log 2.96±0.13. Ayaz. (2008), obtained an average TC removal of 94% (log 1.22) in a horizontal flow CW reactor planted with *Cyperus* and *Lemna*, by varying the HRT from 1.62 to 8.75 days. Tanner et al. (1995), achieved 90 to 99% FC removal (log 1.0 to log 2.0) in subsurface flow horizontal CW planted with *Schoenoplectus validus*, with varied HRT from 2 – 7 days. Decamp et al. (1999), reported 99.75% removal (log 2.6 unit) of coliforms in a subsurface flow CW planted with *Phragmites australis*. Quiñónez-Díaz et al. (2001), obtained 99.99% removal (log 4.0) of FC and TC while treating domestic wastewater in a horizontal CW planted with *Scirpus olneyi*.

One major factor for the inactivation of pathogens in an MFC system is prevailing anaerobic conditions near anode with low ORP (-50 to -125 mV) (Ieropolous et al., 2019). In the present study, reactors R1, R2, and R3 prevailed in anaerobic conditions at the bottom of

Table 2.3 Mean coliform concentration in reactor influent and effluents of R1, R2, and R3

Sample	Total coliform (MPN/100 mL)	Fecal coliform (MPN/100 mL)
Raw wastewater	(12.06±5.78)E+06	(8.87±5.86)E+06
R1 effluent	(13.09±5.42)E+03	(6.98±3.31)E+03
R2 effluent	(14.41±7.23)E+03	(2.79±1.75)E+03
R3 effluent	(13.88±5.11)E+03	(6.25±3.65)E+03

Data format: XX ± YY, where ‘XX’ is the mean, ‘YY’ is the standard deviation the reactors with low ORP (-80 to -88 mV), which was responsible for the removal of coliforms to some extent. In reactor R2, the anode is provided at the bottom, and EABs were also present and active, which is evident from electricity production. One probable reason for enhanced fecal coliform removal in R2 might be the interspecies competition between EABs and pathogens. Table 2.4 shows a comparative study of coliform removal among the current and previous studies. It is seen from Table 2.4 that higher coliform removal in conventional CW systems can only be achieved either with longer HRT or by using multiple CW reactors in series, which results in a higher areal footprint of CW. In the current study, more than 99% coliform removal has been achieved with an HRT of 36 hours, particularly reactor R2 was found to be highly efficient for FC removal. This HRT is less as compared to the other literature which shows that integrated CW-MFC systems can be effective in FC removal without the requirement of higher HRT or area.

In 2004, the Ministry of Urban Development, India; recommended a maximum permissible limit for FC to be 2500 MPN/100mL for discharging domestic wastewater in rivers (Seth, 2016). In reactor R2, the lowest effluent FC concentration was observed to be 200 MPN/100mL during summer. During winter the lowest concentration observed was 1700 MPN/100mL. This shows the capability of planted CW-MFC used in the current study to reduce the fecal coliform load to comply with recommended discharge standards.

Total and fecal coliform removal in R1, R2, and R3 reactors during summer and winter seasons have been summarized previously in Table 2.2. The first-order kinetic model (equation 2.6), was used for this purpose considering the reactors as ideal plug flow (Sperling and Paoli, 2013), as it is said to be the best fit for decay analysis of coliform bacteria in wetland systems (Avelar et al., 2014). The values obtained for TC and FC from all reactors were analyzed to determine the first-order removal rate coefficient (K) after 36 h for total coliforms (K_{TC}) and fecal coliforms (K_{FC}). The effluent TC and FC values for reactors R1, R2, and R3 effluents are illustrated in Figures 2.4 (a), 2.4 (b), and 2.4 (c), respectively. The effect of seasonal temperature variation on the K values was also studied.

In reactor R2, the removal rate coefficient for total coliform (K_{TCR2}) was found in the range of 3.13 to 5.47 d^{-1}, and for fecal coliform (K_{FCR2}) was found in the range of 3.99 to 6.67 d^{-1}. In reactor R1, the coefficient values for total and fecal coliform removal (K_{TCR1} and K_{FCR1}), were found to be within 3.71 to 5.47 d^{-1}, and 3.22 to 5.99 d^{-1}. For reactor R3, K_{TCR3} varied from 3.13 d^{-1} to 5.25 d^{-1}, and K_{FCR3} varied between 3.34 d^{-1} to 6.11 d^{-1}. It was observed that an increase in ambient temperature during summer (March to November) resulted in higher K values for FC removal in the reactors. During winter (December, January, and February) the K values were reduced significantly. This variation is shown in Figure 2.4 (d). The mean K values obtained throughout the study period were, K_{TCR1} = 4.82±0.60 d^{-1}, K_{FCR1} = 5.12±0.63 d^{-1}, K_{TCR2} = 4.83±0.59 d^{-1}, K_{FCR2} = 6.03±0.85 d^{-1}, K_{TCR3} = 4.59±0.48 d^{-1}, and K_{FCR3} = 4.82±0.56 d^{-1}.

Table 2.4 Summary of coliform removal performances by wetlands

Coliform	**Wastewater**	**CW reactor**	**HRT (days)**	**Plants**	**Log removal**	**Reference**
Total coliform	Domestic wastewater	Vertical CW-MFC	1.5	*Typha angustifolia*	3.18	Current study
	Domestic wastewater	HSSF	3	*Eriochloa aristata*	1.79	Caselles-Osorio et al., 2011
	Domestic sewage	HF + VF	2	*Cyperus sp.*	1.54	García et al., 2013
	Domestic wastewater	HF + VF	3	*Scirpus lacustris*	2.7	García et al., 2008
	Tertiary wastewater	HSSF	1.62-8.75	*Cyperus sp.*	1.22	Ayaz, 2008
	Dairy wastewater	HF + VF	10	*Phragmites australis*	2.56	Mantovi et al., 2003
Fecal coliform	Domestic wastewater	Vertical CW-MFC	1.5	*Typha angustifolia*	3.95	Current study
	Domestic wastewater	HF + VF	3	*Scirpus lacustris*	2.88	García et al., 2008
	Domestic wastewater	HSSF	2	Mixed plants	1.8	Neralla and Weaver, 2000
	Domestic wastewater	HF + VF	3	*Cyperus sp.*	1.8	García et al., 2013
	Domestic wastewater	VF + VF	3	*Cyperus sp.*	3.5	García et al., 2013
	Municipal wastewater	HSSF	1	*Salix atrocinerea*	0.98	Ansola et al., 2003

HSSF: Horizontal subsurface flow, VF: Vertical flow, HF: Horizontal flow

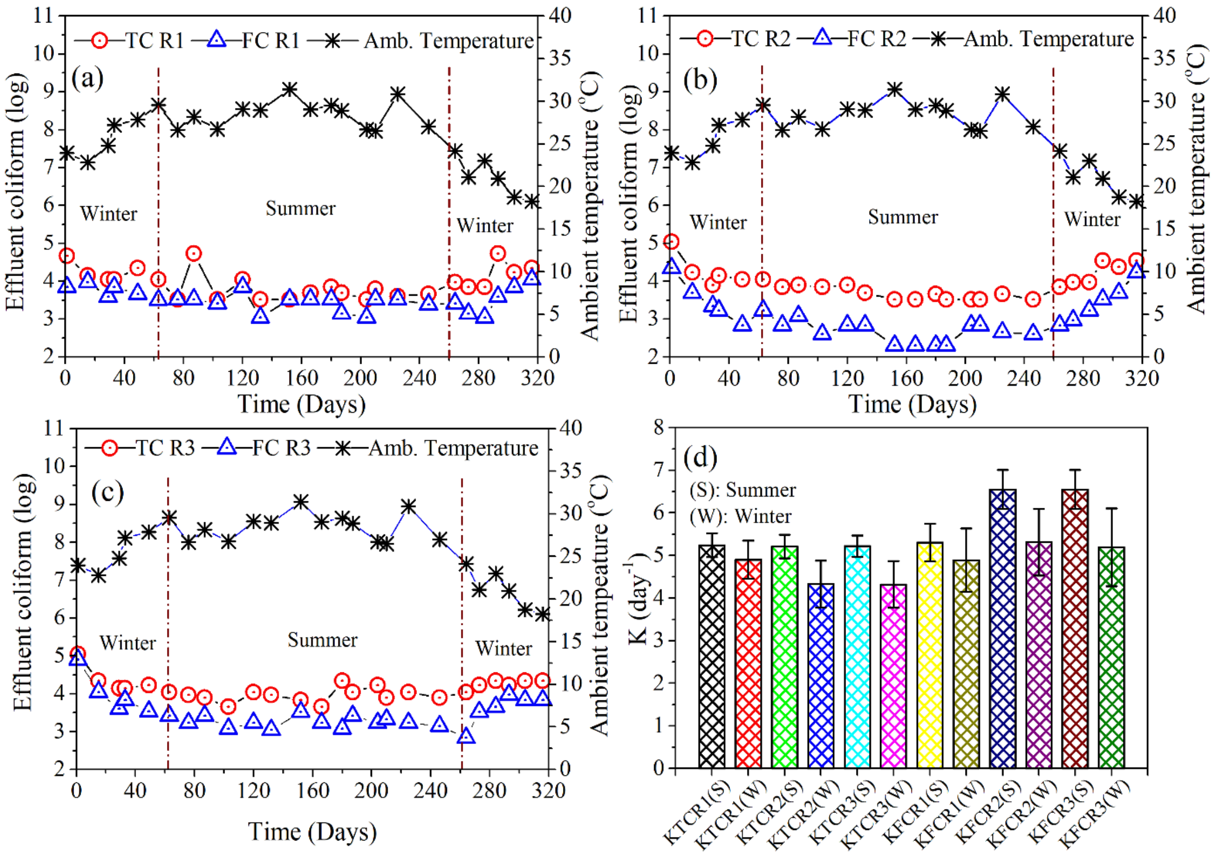

Fig. 2.4 Variation in the effluent for TC and FC in (a) reactor R1, (b) reactor R2, (c) Reactor R3, and (d) comparison of first order FC and TC removal coefficients of the reactors during summer and winter

In the majority of the pieces of literature, the first-order removal rate coefficient for total or fecal coliform is not reported. Most of the available studies where it is reported have been carried out in horizontal flow constructed wetland reactors. Avelar et al. (2014), investigated coliform removal from raw domestic sewage using horizontal subsurface flow CW, and reported a maximum K value of 3.38 d^{-1} for the removal of total coliform and E. coli, in a reactor planted with *Mentha aquatica* with a HRT of 4.5 – 6 days. Jamieson et al. (2007), reported a K value of 0.08 to 0.11 d^{-1} for fecal coliform removal from livestock wastewater using a horizontal flow CW planted with *Typha latifolia*. Boutilier et al. (2009), observed the rate coefficient in the range of 0.02 to 0.03 d^{-1} while treating raw sewage in horizontal flow CW planted with *Typha latifolia* with an HRT of 25 days.

In the current study, more than 99% coliform removal has been achieved with an HRT of 36 hours, which has resulted in much higher values for the first-order removal rate coefficient compared to the literature (especially K_{TCR2} and K_{FCR2}). Particularly R2 was found to be highly efficient for FC removal. However, the obtained K values are reactor-specific as they represent the combined coliform removal effects of multiple mechanisms such as adsorption, natural die-off, sedimentation, filtration, etc. It is seen from Table 2.2 that, during the summer BOD_5 and COD removal performance of all the reactors was increased compared to winter which is possibly due to enhanced heterotrophic activity of the microbes in the raw domestic wastewater. However, the increase in BOD removal efficiency in summer was higher compared to COD removal efficiencies in R1, R2, and R3.

2.3.5 Monitoring of open circuit voltage and operating voltage

Open circuit voltage (OCV) and the operating voltage (OV) of R2 and R3 reactors were measured throughout the operational period. Figure 2.5 (a) shows the OCV variation for R2 and R3 throughout the study. The output OCV observed in R2 and R3 during winter and summer is given in Table 2.2. The positive effects of employing wetland macrophyte in the integrated CW-MFC (R2), for energy harvesting, were visible in the current study. Oon et al. (2016), operated a CW-MFC reactor with external aeration while treating synthetic domestic wastewater. The maximum OCV obtained was in the range of 450-475 mV after 150 days of continuous reactor operation and application of artificial aeration in the cathode region. The influent COD was 624 mg/L and the maximum removal efficiency was observed to be 99%. Another study by Das et al. (2020), reported a similar pattern of OCV generation in an MFC using anaerobic septic tank sludge as anode inoculum and providing the cathode chamber with external aeration. The stability in voltage output was achieved after 40-50 days. The maximum OCV was 500-550 mV. The maximum COD removal efficiency was found to be 87.70±4.50% with an influent COD level of 2987±435 mg/L. Fang et al. (2015), operated a vertical up-flow CW-MFC reactor with an average influent COD level of 135±10 mg/L, and the maximum OCV obtained was 510 mV with an HRT of 72 hours without any artificial aeration. The corresponding maximum COD removal was 85.66%. In the current study, R2 achieved higher OCV output while achieving more than 90% COD removal from low-strength domestic wastewater without external aeration, compared to the literature. This shows that *Typha angustifolia* can be an interesting alternative for artificial aeration in a CW-MFC system for enhanced bioelectricity production.

2.3.6 Role of plants in enhanced bioelectricity generation

Growth of the macrophytes in both R1 and R2 reactors was monitored in the current study along with the comparison of OCV production in R2 and R3 to check the positive effects of

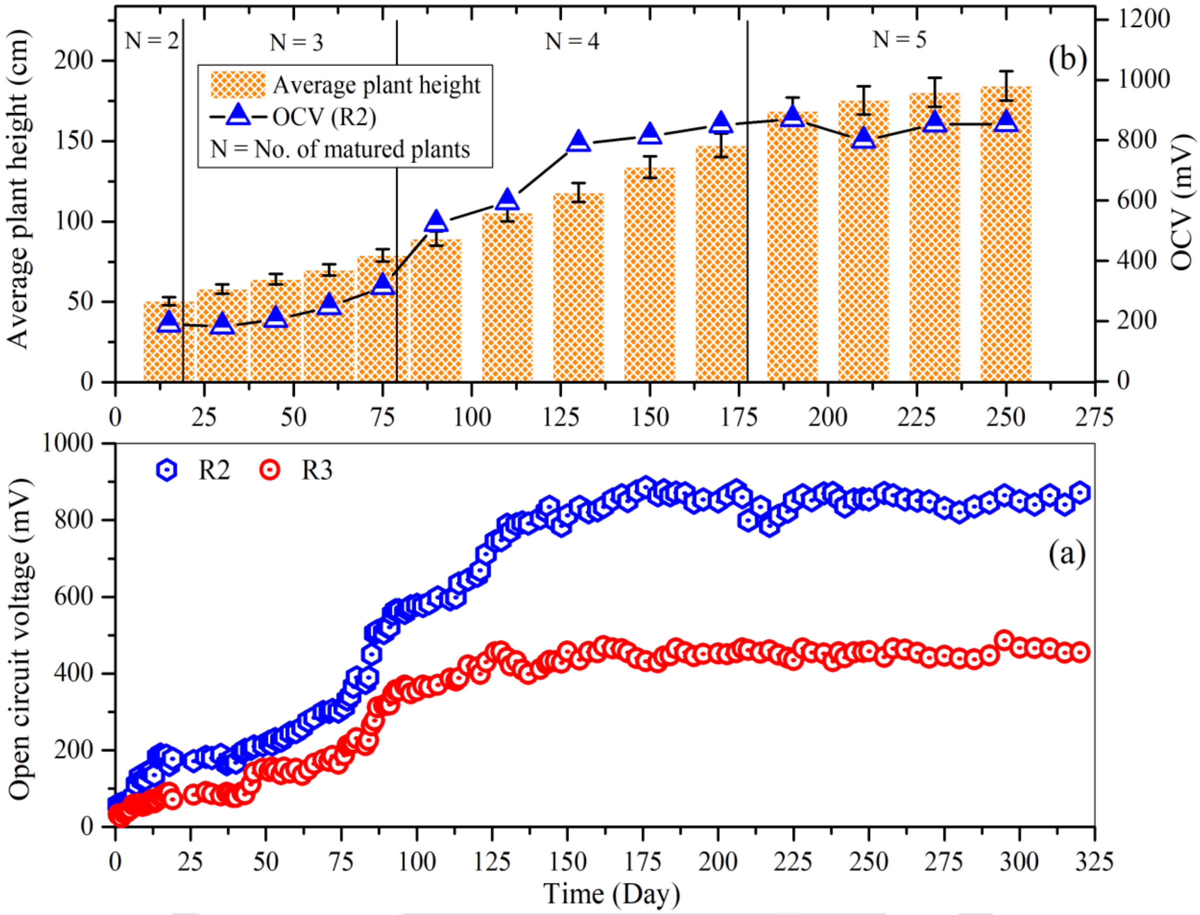

Fig. 2.5 Monitoring of (a) OCV generation in R2 and R3 reactors during the study and (b) monitoring of plant growth with OCV production in reactor R2

plants in bioelectricity enhancement. The result of plant growth in terms of number of plants and average height has been summarized in Table 2.5. After the plantation of macrophytes in R1 and R2, not much growth was observed during the first 20 days. After 20 days, new shoots were noticed. Since then the macrophytes in reactors R1 and R2 have shown good growth in number and the count of matured plants were doubled (from 2 to 4) between 70 to 90 days. This positive growth rate in stem number, height, and number of leaves per plant indicates that the plants were well acclimatized to raw wastewater and are capable of reproduction under experimental environmental conditions. Growth patterns of the plants were found to be similar in R1 and R2 reactors as for both of them, the feed wastewater and ambient environment were similar.

Figure 2.5 (b) shows the plant growth with OCV monitoring in reactor R2. From the plot, it is seen that the maximum growth rate in average plant height and OCV was observed simultaneously between 75 to 175 days with 4 matured plants in the reactor corresponding to a stem density of 140 per m^2 area. From 175 days to 250 days, a decline in the rate of plant growth and OCV was observed even though the number of plants was increased to 5. The simultaneous increase in OCV generation with the growth of macrophytes in reactor R2 suggests the plants' contribution to increased bioelectricity production. The positive contribution of different species of wetland macrophytes for the enhancement of bioelectricity production in CW-MFC is also well-established in the literature (Yang et al., 2021; Zhu et al., 2021). The higher OCV output in R2 compared to R3 can be solely attributed to the *Typha angustifolia* plants as other factors like organic loading, pH, ambient temperature, anolyte characteristics, etc. were identical in both reactors. An increase in the average height and number of plants in R2 might have influenced a further increase in the redox potential due to an increase in root density and resulted in the continuous rising trend and higher OCV output (Dong et al., 2011). The increase in voltage in reactor R2 can be attributed to the increased redox potential at the cathode provided by the roots of *Typha angustifolia* (Dong et al., 2011; Wießner and Kuschk, 2002).

2.3.7 Reactor polarization power density and coulombic efficiency

The polarization study was performed after both the reactors (R2 and R3) reached a steady state in terms of OCV production, i.e. after 180 days of continuous operation. The Polarization curves for both R2 and R3 as shown in Figure 2.6 (a), were obtained by connecting the range of resistances (1Ω to 1MΩ) to the external circuit and measuring the OV at resistor ends. The internal resistance of R1 and R3 reactors were 310 Ω and 312 Ω respectively as was obtained from the slope of the operating voltage vs current plot in Figure 2.6 (b). The variation of power density with current density for both reactors has been illustrated in Figure 2.6 (a). The maximum power density (PD) and current density (CD) in reactor R2 were observed to be 4.65 mW/m^2 and 31.88 mA/m^2, which are considerably higher than the R3 reactor, 1.32 mW/m^2, and 16.58 mA/m^2. One of the primary factors that affect the power density of an MFC is the distance between the anode and the cathode. In the current study, this distance for both reactors being the same supports the fact that CW-MFC has the capability of enhancing the power output (Yang et al., 2021).

Coulombic efficiency (CE) is a representative parameter of electron conversion from the available organic matter in bioelectrochemical systems. In the current study, CE was also determined after 180 days of reactor operation. The mean values of CE in R2 and R3 reactors were found to be 0.93±0.06% and 0.55±0.04% respectively with maximum CE measured as 1.01% for reactor R2 and 0.63% for reactor R3. Lower CE is an indication of less amount of

Table 2.5 Plant growth measurement in reactor R1 and reactor R2

Time (days)	R1 (CW)		R2 (CW-MFC)	
	Plant number	Average height	Plant number	Average height
15	2	49.98 cm	2	50.51 cm
30	3	57.77 cm	3	58.01 cm
45	3	62.36 cm	3	64.12 cm
60	3	69.65 cm	4	69.95 cm
75	3	79.65 cm	4	78.98 cm
90	4	91.36 cm	4	89.36 cm
110	4	106.56 cm	4	105.39 cm
130	4	119.36 cm	4	118.08 cm
150	4	133.51 cm	4	133.87 cm
170	5	146.36 cm	5	147.41 cm
180	5	169.78 cm	5	168.65 cm
200	5	176.45 cm	5	175.43 cm
220	5	179.36 cm	5	180.33 cm
240	5	183.56 cm	5	184.36 cm

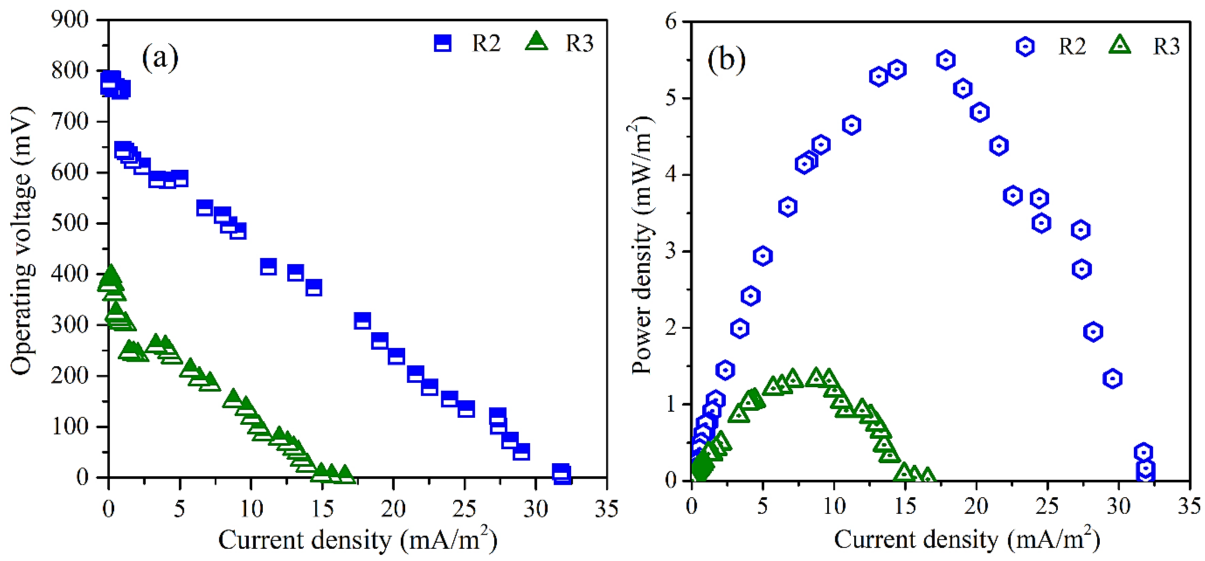

Fig. 2.6 (a) Polarization plot and (b) power density and current density plot for R2 and R3

electron transfer to the anode by the EABs during the decomposition of the soluble organic matters present in the system. Higher CE in the R1 compared to the R2 may be an indication of more EAB activity in the integrated reactor (Yakar et al., 2018). The exclusion of membrane separators in both reactors may also have contributed to the reduced CE values (Fan et al., 2007). The CE for reactor R2 in the current study was comparable with the studies previously conducted by Yakar et al. (2018), and Wang et al. (2016). The reported CE values were 1.42% and 1.64%. However, in some previous studies, the reported CE values were significantly higher such as 6.67% (Srivastava et al., 2020), and 14.90 % (Liu et al., 2017). Multiple factors can influence the electrical performance of a CW-MFC, like electrode material, surface area and volume of the anode, the distance between the electrodes, initial organic strength of the wastewater, redox gradient, and the dominance of EABs in the anode chamber over the heterotrophs. To get higher CE from a CW-MFC it is desirable to maintain or create favorable environmental stress conditions to make EABs the dominant species. However, the primary concern of wastewater treatment is always the efficient removal of organics and pathogens to facilitate reuse.

2.4 Statistical analysis of the data

The data related to the effluent quality of different reactors were statistically analyzed using single-factor ANOVA analysis with MS-Excel software version 16. This test was performed to ensure that the integrated CW-MFC reactor (R2) was able to produce an effluent that bore significant qualitative differences from CW (R1) and unplanted CW-MFC. COD and BOD_5 were selected as the effluent quality parameters for ANOVA analysis. The analysis has revealed that effluent BOD_5 of reactor R2 was not significantly different from R1 and R3 with $p > 0.05$. However, the ANOVA analysis for effluent COD has exerted a significant difference in R2 effluent, compared to the R1 and R3 reactors. The results of the ANOVA analysis have been summarized in Appendix A, Table A1, Table A2, Table A3, and Table A4, respectively.

2.5 Conclusion

Three lab-scale reactors with identical dimensions but distinct configurations have been operated and monitored for the treatment of low-strength domestic wastewater. The planted CW-MFC reactor (R2) was found to be more effective for wastewater treatment in terms of organic and coliform removal compared to the other two reactors. All three reactors were tolerant to the fluctuations of actual domestic wastewater compositions and exerted similar biodegradable organic removal efficiency as BOD_5. Higher removal of the refractory organics was noticed in the planted CW-MFC reactor as compared to the constructed wetland (R1) and unplanted CW-MFC reactor (R3). These findings affirm the combined contribution of plants and electrodes in integrated systems for better COD removal efficiency. Voltage generation, maximum current density, and power density were observed to be higher in reactor R2 compared to reactor R3. This shows the plants' positive contribution to bioelectricity augmentation. Another major finding of the study was the enhanced fecal coliform (FC) removal capability of reactor R2, compared to R1 and R3. FC inactivation and organic removal were found to be affected by the seasonal temperature variations to considerable levels. The current findings warrant further research to investigate the inherent mechanisms of the integrated CW-MFC reactor for enhanced FC and COD removal. This could be facilitated by assessing the microbial population inside the reactor to see any dominance of EABs in the bacterial pool, electrode-EAB interaction, potential role of redox conditions inside the reactor, spatial characterization of reactor effluents, and kinetic analysis of FC inactivation under different reactor operating conditions.

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Chapter 3

Investigation of Coliform Removal Mechanism in CW-MFC System: Spatial Characterization and Microbial Population Assessment

This chapter is primarily focused on the investigation of the inherent pollutant removal mechanism of the integrated CW-MFC reactor with a particular emphasis on the coliform inactivation pathway. This chapter also deals with the assessment of the microbial population inside three different reactors used in this study and the spatial analysis of multiple biological and physicochemical parameters to understand the working principle of these systems.

3.1 Introduction

The major pollution in raw domestic wastewater is due to dissolved organics and fecal coliforms (FC) or pathogenic microorganisms (Wu et al., 2016). In the past, multiple studies have investigated the application of CW-MFC for synthetic wastewater treatment but the major shortcoming of using synthetic domestic wastewater is it does not account for the FC removal capabilities of the reactors which restricts the direct reuse of treated wastewater. Some studies in the past have investigated the capacity of coliform bacteria removal in autonomously operated constructed wetlands (CW) where it is established that high hydraulic retention time (HRT) is necessary for achieving a desired level of coliform removal (Jasper et al., 2013, Lightbody et al., 2008). Few amounts of studies are documented in the literature related to the investigation of FC removal efficiency and mechanism in integrated CW-MFC reactors. In the previous chapter, a higher amount of organic and FC removal in an integrated CW-MFC reactor, compared to an identical standalone CW reactor while long-term treatment of low-strength raw domestic wastewater laden with FCs has been documented. Previously, some studies have investigated the fate and removal of pathogenic bacteria in the cascade MFC systems, along with the mechanism of pathogenic microbe inactivation by anolyte and catholyte combinations (Ieropoulos et al., 2017; 2019). Some recent research was also focused on investigating coliform removal from domestic wastewater using CW-MFC technology

(Karla et al., 2022, Colares et al., 2021). However, the inherent mechanism of FC removal in a CW-MFC reactor is yet to be well understood. It is also important to explore any effect of the dominating electroactive bacteria (EAB) population inside the reactor and higher EAB activity near the anode on enhanced FC removal.

In this chapter, the investigation of the pathway of organics and fecal coliform removal in an integrated CW-MFC system was done by assessing the spatial distribution of the physicochemical and biological parameters inside the reactors. Assessment of the microbial population in individual systems was performed to get a better insight into the effects of the electrodes and the plants on biomass diversity. A biomass activity study was performed to assess the role of microbial degradation in the reactors. Different batch studies were performed to understand if any positive role is played by EABs in higher fecal coliform removal in the process of bioelectricity production via EAB-anode interaction.

3.2 Materials and methods

The materials used for this phase of research, including the raw wastewater, wetland macrophyte (*Typha angustifolia)*, reactor configuration and media, electrode arrangements, and hydraulic retention time of the reactors were similar to those described in Chapter 2, Section 2.2.2. The reactor designations are also identical to Chapter 2, such as the constructed wetland is named R1, the integrated CW-MFC reactor is designated R2, and the unplanted CW-MFC is designated R3. All the reagents used in the current study for chemical analysis of the raw wastewater and reactor effluents were of analytical research (AR) grade. The schematic diagram of the reactor configuration along with the plants and electrode arrangements have been shown in Figure 2.1 of Chapter 2.

3.2.1 Batch studies

Multiple batch experiments were performed to determine the first-order fecal coliform (FC) removal rate coefficient (K) under different experimental conditions. The obtained results were analyzed to find out the K value Equation 2.6 in Chapter 2, and the slope of the kinetic analysis plots, under different combinations of factors. The time frame of these studies was kept up to 36 hours corresponding to the reactor hydraulic retention time (HRT). The first batch study was performed with raw wastewater to check the natural die-off coefficient of FC. Raw wastewater was kept in 250 mL serum bottles having provisions for gas release and sample collection. Bottles were incubated at a summer average temperature of 31.0 °C and a winter average temperature of 23.4 °C as was observed in Chapter 2. One set of samples was purged with nitrogen gas after sample collection to ensure anaerobic conditions during the complete batch study. Another set of samples was kept without purging.

In the second batch study, reactors R2 and R3 were operated in batch mode for successive open-circuit and closed-circuit modes for 36 h. During the second batch study, open circuit voltage (OCV) was monitored for both R2 and R3 reactors to get an indication of EAB activity at the anode. While R2 and R3 were operated in closed circuit mode, the source voltage (also termed OCV) was measured at the end of the graphite rods extended from the anode and the cathode; by temporarily removing the external resistance. In the second batch study, samples were analyzed from sampling port S1 in all three reactors for the quantification of fecal coliforms.

In the third batch study, raw domestic wastewater was inoculated with anolyte obtained from R2 and R3 with two different dilution factors (D_f) of 8 and 4, to check the effect of active EABs on fecal coliforms without the presence of any external electron acceptor. During the 3rd batch study, the incubator temperature was kept at 31.0 °C, which is the mean summer temperature.

3.2.2 Analytical methods

Effluents were obtained periodically from all the reactors (R1, R2, and R3) for analyzing BOD_5, COD, pH, total and fecal coliforms, total suspended solids (TSS), total dissolved solids (TDS), volatile suspended solids (VSS), and redox potential (ORP). The hydraulic retention time was kept as 36 h in all reactors. The bottom-most sampling port in the reactors was termed S1 (near the anode region of reactors R2 and R3 and 12 cm from the substratum). Sampling port S2 was kept at a height of 20 cm from the substratum (between the anode and the cathode in reactors R2 and R3), and the top-most effluent collection port was named S3 which was placed 30 cm above the substratum (near the cathode region in reactors R2 and R3). Analysis of BOD_5, COD, pH, DO, ORP, total coliform (TC), and fecal coliform (FC) were carried out using the processes described in section 2.2.4 of Chapter 2. The heterotrophic plate count test was performed by the pour plate technique. Ammonia was determined by the phenate method. All the laboratory analyses were performed in a triplicate format and adhered to the experimental protocols given in the standard methods (APHA, 2017). The kinetic batch analysis for FC removal was performed in a cycle of three for each set of experiments to ensure the repeatability and accuracy of the results obtained. Calculation of HRT, organic removal efficiency, log removal of TC and FC, and the first-order removal rate coefficient for coliform inactivation were performed using equations 2.1, 2.4, 2.5, and 2.6 respectively as described in Chapter 2.

3.2.3 Biomass activity study

The heterotrophic biomass activity assays were performed to determine the specific substrate utilization rate by reactor biomass. Initial VSS was measured by extracting gravel media (as attached biomass) from the reactors and agitating with purged deionized water (50 mL) for 2 h. Volatile suspended solid (VSS) was calculated after filtration of the purged water. An initial biomass of 1.25 – 1.4 g L^{-1} was applied at a similar ratio as was available in the reactors. The batch activity study was conducted in 1 L serum bottles with 500 mL liquid volume, air-tight capping, and provision for sample collection and gas release. Acetate was used as the sole carbon source to maintain an initial soluble COD of 330 to 335 mg/L. Nutrients were not supplemented to limit biomass growth during the activity assay inside the batch reactors. At the start of every cycle, batch reactors were purged for 10 minutes, and at t = 0, the samples were collected using a suction syringe. The reactors were continuously stirred and sampling was done at short intervals (0.25–1 h) followed by elongated intervals (2–6 h) up to 36 h, for the first cycle. The study was continued for three cycles. After completing the preceding cycle, the supernatant was slowly decanted and then refilled with fresh feed. After completing cycle 3, the final VSS was measured. Cycle 1 and cycle 2 stand for the biomass acclimation period. Biomass activity was determined by Equation 3.1, given the linear portion (slope) of cycle 3 (Singh and Chakraborty, 2022).

$$\text{Biomass activity}\left(\frac{\text{mg COD removed}}{\text{mg VSS d}}\right) = \text{slope}\left(\frac{\text{mg COD removed}}{\text{mg VSS d}}\right) \times \frac{24\ h}{d} \times \frac{1}{\text{mg VSS}} \tag{3.1}$$

3.2.4 Analysis of microbial metagenomics

Metagenomic analysis of the microbial pool was performed for R1, R2, and R3 reactors; to assess the difference in the dominant microbial population inside the reactor control volume. The taxonomic profiles of microbial communities in reactors R1, R2, and R3 were analyzed using 16S Microbiome Profiling. Homogenous wastewater samples (as representative samples for individual reactors) were obtained from all the reactors and were sent to Eurofins Genomics (Bengaluru, India) for 16S (V3–V4) ribosomal RNA (rRNA) sequencing using the Illumina

MiSeq platform. To prepare the representative sample, reactor media was collected from the location of every sampling port of the reactors and was mixed with 50 mL of deionized water using a vortex mixer for 5 minutes to make it homogeneous. The obtained metagenomics results for different reactors have been deposited into the National Centre for Biotechnology Information (NCBI) database. The genome sequencing data of Reactor R1 and Reactor R2 are available in the bioproject ID PRJNA820999 with biosample accession numbers SAMN27035155 and SAMN27035156 respectively. The genome sequencing results of Reactor R3 are available in the bioproject ID PRJNA984182, with biosample accession number SAMN35742750.

3.3 Results and discussion

The findings of this phase of the study on the spatial characterization of reactor effluents and organics and FC removal mechanisms with a brief discussion of the obtained results have been summarised in the following sections of this chapter as follows:

3.3.1 Spatial variation of BOD_5 and COD during the study

Figures 3.1 (a) and 3.1 (b) show the mean effluent BOD_5 and effluent COD obtained from the sampling ports of different reactors. It was observed that around 65% – 70% BOD_5 removal was obtained at sampling port S1 and 70% – 80% BOD_5 removal was achieved at S2 in all the reactors. The distance from the inlet to sampling ports S1 and S2 consumed around 30% and 50% of the reactor length. This high BOD_5 removal within 50% of the reactor length is indicative of the high biodegradability of the wastewater. During the study, the BOD_5/COD ratio for the wastewater feed was found to be 0.57±0.03. Low organic strength is also responsible for a considerable amount of BOD_5 reduction within the distance of the S2 port. Overall BOD_5 removal in both planted and unplanted reactors was around 87%. The results suggested that all the reactors were highly efficient for BOD_5 removal, and the contribution of plants and the electrode arrangement were negligible.

The average effluent COD of R2S1 (reactor R2 and sampling port S1) was less than R1S1 and R3S1. Mean COD removal obtained at R2S1 was 60% – 65%, compared to 45% – 52% at R1S1, and 56% – 61% at R3S1 (within 30% of reactor length). Around 80% – 85% COD removal was achieved at R2S2 (within 50% of reactor length), compared to 62% – 70% in R1S2, and 70% – 76% in R3S2. The spatial variation of the BOD_5/COD ratio showed the

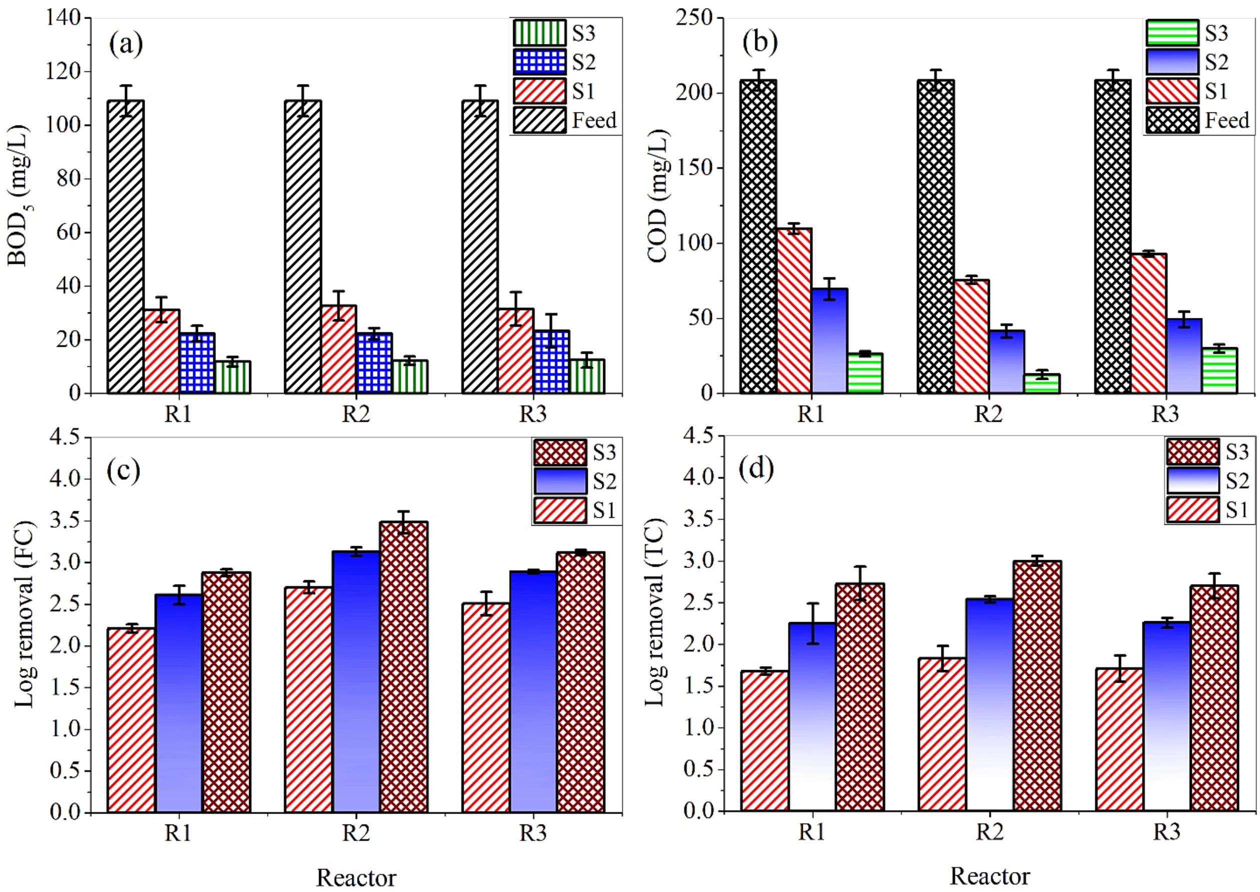

Fig. 3.1 (a) Spatial distribution of effluent BOD_5 and (b) effluent COD as obtained from the sampling ports of different reactors, (c) cumulative log removal of FC, and (d) TC as obtained from the reactors

increasing trend from sampling port S1 to port S3 in all reactors. At R1S1, R1S2, and R1S3; the BOD_5/COD ratio was 0.28±0.04, 0.32±0.06, and 0.46±0.08. R2S1, R2S2, and R2S3 have shown a BOD_5/COD ratio of 0.43 ±0.07, 0.54±0.11, and 0.71±0.13 respectively. In case of R3, BOD_5/COD ratio was found to be 0.34±0.07, 0.48±0.16, and 0.42±0.11, for S1, S2, and S3. In the study, integrated planted CW-MFC (R2) showed better COD removal compared to planted CW (R1) and unplanted CW-MFC (R3) throughout the length of the reactor (Figure 3.1 b). This resulted in lower effluent COD in the ports of R2 compared to R1 and R3 and the results are also reflected in the BOD_5/COD ratio of the reactors. BOD_5 represents the biodegradable soluble organic fraction in the wastewater and COD combines the bio-refractory fraction of soluble organics with the biodegradable portion which could be chemically oxidized by strong oxidizing reagents lime permanganate or dichromate. Higher COD removal could be associated with EAB-anode interaction that allows better external electron transfer at the anode which was plausible for R2 and R3 but not in R1. Better COD removal obtained at R2S1 compared to R3S1 is a result of higher EAB activity in R2 due to higher cathode potential (Gupta et al., 2023; Liu et al., 2022). The current findings are in agreement with the findings of Ramírez-Vargas et al. (2019), where planted integrated electrochemical systems performed better for COD removal but not for BOD_5.

3.3.2 Spatial variation of total and fecal coliform removal efficiency

Figures 3.1 (c) and 3.1 (d) illustrate the spatial cumulative removal of fecal coliforms (FC) and total coliforms (TC) in reactors R1, R2, and R3. Coliform removal is referred to as the reduction in MPN (most probable number) counts in the effluent, compared to the raw wastewater. The data have been reported in a log scale for a better and more concise representation. R2S1 exerted maximum TC and FC removal compared to that of R1S1 and R3S1 which is a probable result of prevailing anaerobic conditions combined with active EABs (Jasper et al., 2013; Ieropoulus et al., 2019). The increasing level of coliform removal from S1 to S3 might also be a result of less organic availability towards the cathode from the inlet as observed in Figures 3.1 (a) and 3.1 (b). Results have shown that the majority of the coliforms are removed within the bottom half of the reactors, suggesting the effectiveness of both anaerobic conditions and EAB-anode interaction for increased coliform removal. Better cumulative FC and TC removal was observed in R2S1 and R2S2 compared to R3S1 and R3S2 affirming the ability of planted CW-MFC for higher coliform inactivation (Kumar and Singh, 2020).

3.3.3 Spatial variation of other physicochemical and biological parameters

Constructed wetlands, microbial fuel cells, and integrated CW-MFCs function as a collective of physical, chemical, and biological processes that lead to pollutant degradation under the same control volume (Liu et al., 2015). These processes are dependent and highly influenced by the physicochemical and biological characteristics of the wastewater and their alteration in concentration inside the reactor. To get a better insight into the working mechanism of R1, R2, and R3, spatial variation of the heterotrophic bacterial population (CFU/100 mL), TSS, TDS, VSS, NH_4^+–N, pH, ORP, and DO were analyzed in all the reactors. The results are illustrated in Figure 3.2. CFU (Fig. 3.3 a) has shown a declining trend from sampling port S1 to S3, in all three reactors due to a reduction in soluble organic content in the wastewater resulting in less availability of food for heterotrophic microorganisms. The sampling ports S1, S2, and S3 in the R2 reactor rendered minimum CFU values (presented in log scale), which is indicative of better organic removal in R2 compared to R1 and R3. A similar declining trend was observed for TSS and VSS in all the reactors, while TDS increased from S1 to S3 (Fig. 3.3 b).

Determination of VSS offers a rough estimation of the amount of organic matter present in a sample. Microbial biomass is mainly composed of organic materials.

Therefore VSS can furnish a rough idea about the biomass content. A declining trend in VSS supports the trend of CFU variation in the reactors. Figures 3.2 (c) and 3.2 (d) show the variation in ammonia concentration and pH throughout the length of the reactors. Ammonia (NH_4^+–N) concentration has reduced from S1 to S3 in planted reactors R1 and R2. The pH variation of the systems would depend on the relative abundance of NH_3 and NH_4^+. Consumption of H^+ ions during the cathode reaction might also increase the pH at S3 up to some extent inside reactors R2 and R3 (Huang et al., 2021). Figures 3.2 (e) and 3.2 (f) show spatial variation in ORP and DO in different reactors. ORP and DO have shown a simultaneous increase in planted reactors (R1 and R2) due to radial oxygen loss by *Typha* plants. Limited rise in ORP is observed at R3S3 which is probably a result of organic decomposition (Zhai et al., 2012).

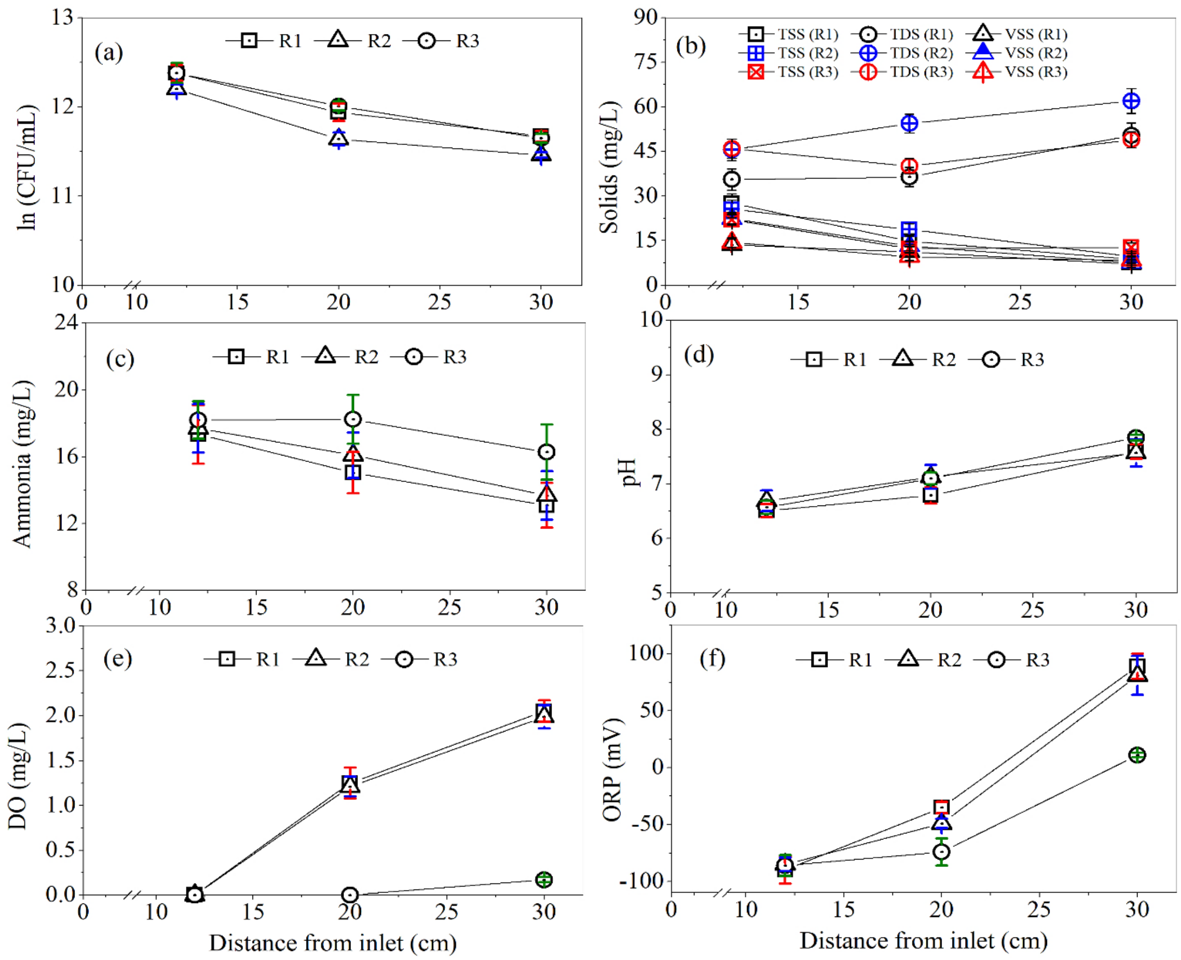

Fig. 3.2 Mean spatial variation of physicochemical and biological parameters in different reactors plotted as distance from the inlet

3.3.4 Assessment of microbial population

The difference in the microbial population in terms of phylogenetic abundance is well observed from the comparative microbial metagenomics results of R1 and R2 reactors. The comparative phylum level and class level analysis of reactors R1 and R2 have been shown in Figure 3.3 and Figure 3.4 respectively. For reactor R1, the microbial population at the phylum level mainly consisted of *Proteobacteria* (50.10%), *Bacteroidetes* (9.20%), *Firmicutes* (7.60%), *Actinobacteria* (12.10%), *Verrucomicrobia* (3.00%), *TM7* (2.60%), *OD1* (1.90%), and *GNo2* (1.70%). *Chloroflexi*, *Nitrospirae*, *Acedobacteria*, *Chlorobi*, *Planctomycetes*, *Spirochaetes*, *Thermi*, *OP3*, *OP11*, and *Crenarchaeota* phyla have shown less than 1.50% abundance individually. In the case of Reactor R2, the abundance of *Proteobacteria* has reduced to 32.50%, and the population of *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* have been increased to 24.80%, 21.20%, and 14.00%, respectively. These microorganisms are well known for their abundance in natural anaerobic environments and their capability of extracellular electron transport (Mani et al., 2020; Burns et al., 2012). The results suggest that the presence of insoluble electron acceptors (anode and cathode) can increase the diversity and dominance of EABs over other microbial populations.

Phylum *Nitrospirae* was also detected in Reactor R1 (1.4%) and R2 (0.9%). *Nitrospirae* is known as aerobic chemolithoautotrophic nitrifying bacteria (Daims and Wagner, 2018). The presence of these microbes also affirms the development of a micro-aerobic environment near the rhizosphere in Reactor R1 and Reactor R2. This fact can also be supported by the minute DO level and high ORP detected at the cathode region (S3) of R1 and R2. In Reactor R1, *alphaproteobacteria* and *betaproteobacteria* dominated the class-level population with a combined abundance of nearly 44%. In reactor R2, class level population is mostly dominated by *Clostridia* (phylum *Firmicutes*), *Bacteroides* (phylum *Bactereodetis*), and *actinobacteria* (phylum *Actinobacteria*) with a combined abundance of approximately 50%.

Assessment of microbial population in Reactor R3, at phylum level and class level, has been illustrated in Figure 3.5. At the phylum level, the population mainly consists of *Proteobacteria* (68.26%), *Bacteroidetes* (27.65%), and *Actinobacteria* (3.77%). The cumulative abundance of other phyla such as OD1, *Firmicutes*, *Nitrospirae*, *Verrucomicrobia*, and *Chloroflexi* was less than 1%. In terms of abundance at the class level, the microbial population inside Reactor R3 was dominated by *Gammaproteobacteria* (46.19%). The profusion of other classes was found to be less than 1% individually. The biomass diversity of unplanted R3 was significantly hampered compared to planted R1 and R2 reactors. This was observed as Reactor R3 did not possess the advantage of a large surface area provided by the plant roots for biomass attachment, growth, and activity. *Firmicutes* are well known for their extracellular electron transfer activities and capabilities for degrading complex carbon (Singh and Chakraborty, 2022; Daims and Wagner, 2018). Reduced abundance of *Firmicutes* and *Actinobacteria* seemed to be the major reason for less COD removal and low bioelectricity production in Reactor R3 compared to Reactor R2 (Ji et al., 2022; Perera et al., 2022). The abundance of *Nitrospirae* was dropped to 0.01% in Reactor R3, justifying the very small amount of ammonia removal in R3, compared to R1 and R2 (Fig. 3.3 c). This also suggests that a higher abundance of *Nitrospirae* was associated with the cathode near the root zone strengthening the probable existence of a microaerobic or facultative environment and some amount of ammonia conversion into nitrate (Bai et al., 2023).

Microbial population assessment of R2 at the genus level also revealed the dominance of EABs, it showed a maximum abundance of an unclassified genus of *Veillonellaceae* family (15.38%) from phylum *Firmicutes*, followed by another unclassified genus (12.04%) from phylum *Bacteroidetes*. This was followed by an unclassified genus from the family *Microbacteriaceae* (10.0%), and the genus *Flavobacterium* (6.13%) belonging to phylum *Actinobacteria*. In the case of reactor R3, the genus-level population was dominated by *Flavobacterium* (24.13%) from phylum *Bacteroidetes*, followed by *Acinetobacter* (23.52%), *Pseudomonas* (14.54%), and unclassified genus of family *Comamonadaceae* (9.57%) belonging to phylum *Proteobacteria* (see Appendix B, Fig. B2 and Fig. B3). In Reactor R1, maximum dominance was observed from an unclassified genus of the order *Actinomycetales* (8.85%) from phylum *Actinobacteria*, followed by an unclassified genus of family *Sphingomonadaceae* (8.75%) and order *Rhizobiales* (5.55%), from phylum *Proteobacteria*. The dominance of *Firmicutes* was observed to be low in R1 at the genus level with the presence of *Bacillus* being only 4.75% (see Appendix B, Fig. B1). The genus-level analysis showed a maximum abundance of *Firmicutes* in R2 amongst all three reactors employed in the current study, which is a probable reason that Reactor R2 showed higher COD removal and FC inactivation compared to Reactor R1 and Reactor R3. High ORP at the cathode of R2 might have helped the growth of *Firmicutes* which resulted in the production of higher bioelectricity compared to Reactor R3.

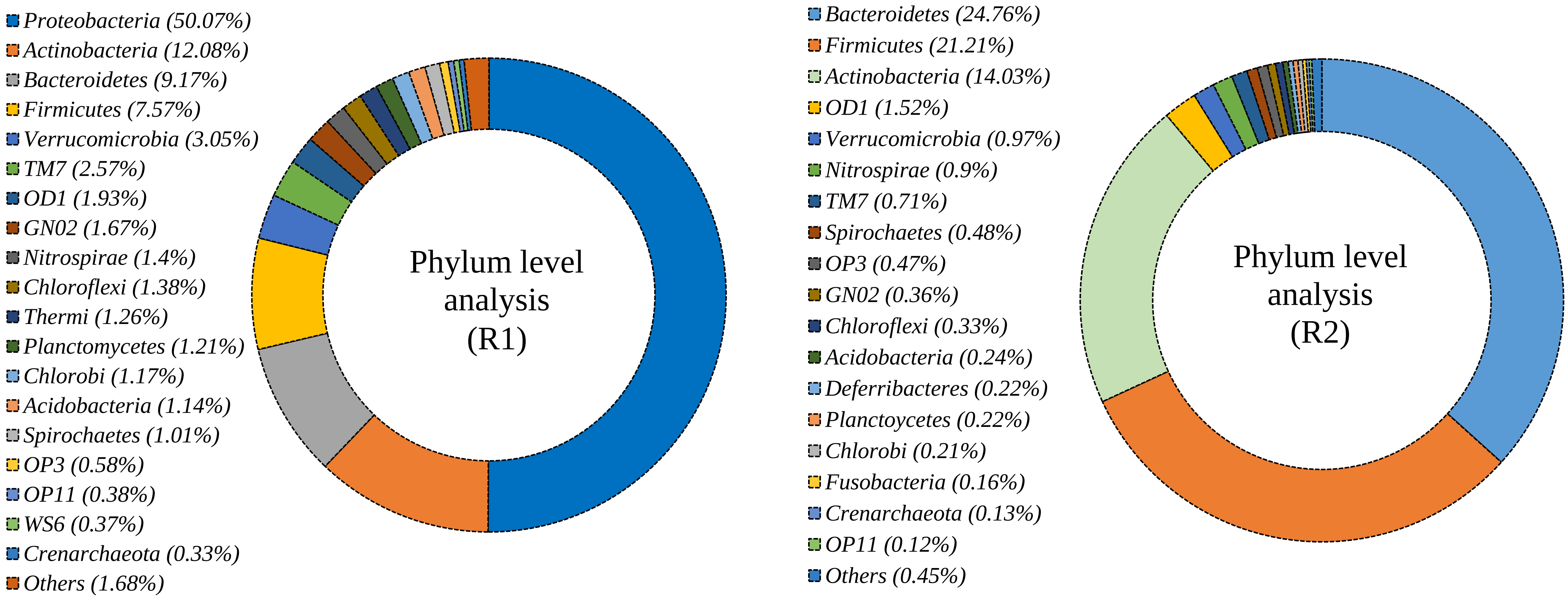

Fig. 3.3 Relative abundance (%) of dominant microbial lineages at phylum level in Reactor R1 and Reactor R2

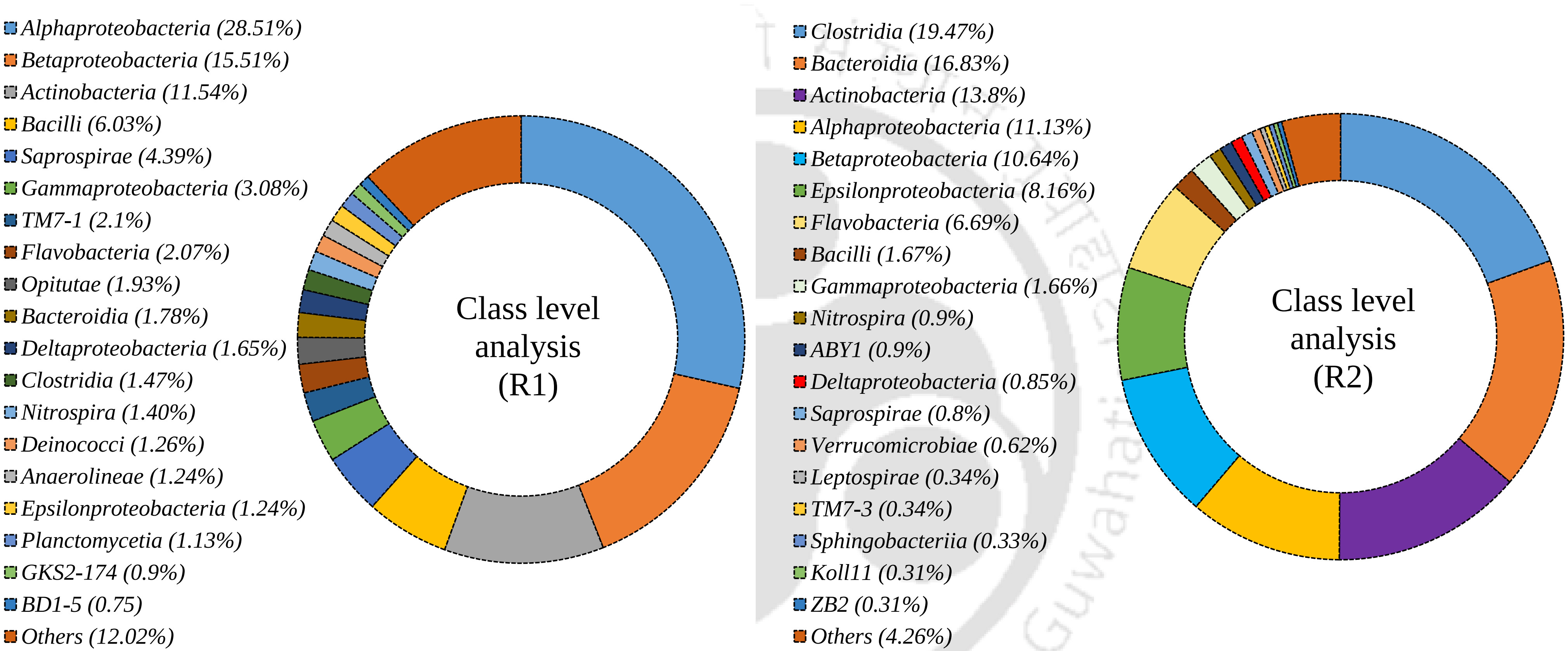

Fig. 3.4 Relative abundance (%) of dominant microbial lineages at class level in Reactor R1 and Reactor R2

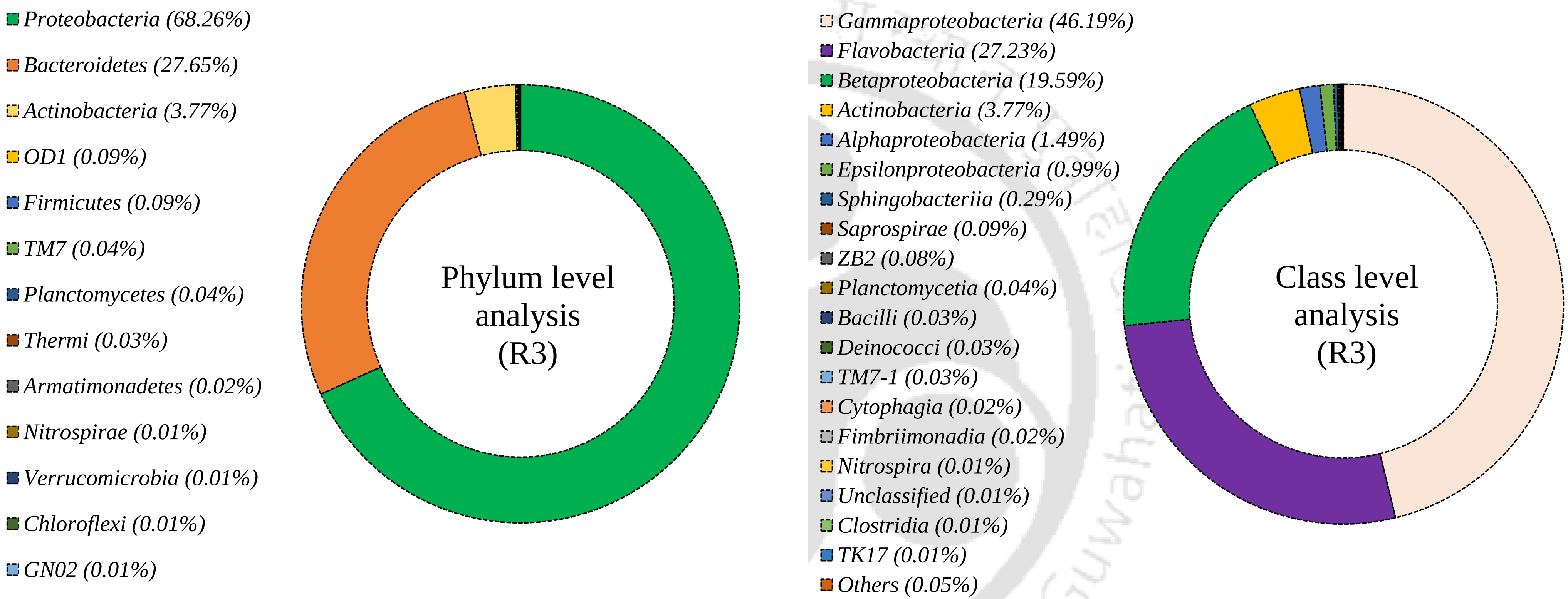

Fig. 3.5 Relative abundance (%) of dominant microbial lineages at phylum level and class level in Reactor R3

3.3.5 Biomass activity study

This study was performed for the assessment of specific substrate utilization rates by active microorganisms present inside reactors R1, R2, and R3 after 320 days of reactor operation. Figure 3.6 shows the biomass activity results of different reactors and the photograph of the three serum bottles used for the batch study arrangement for executing the biomass assay experiments. The heterotrophic activity was determined from the maximum slope of the activity assay, which represented maximum substrate utilization by active biomass (Kumar et al., 2023; Negi et al., 2020). Biomass activity for reactors R1, R2, and R3 was found to be 0.84, 1.27, and 0.93 mg COD mg VSS^{-1} d^{-1}, respectively. R2 showed the maximum biomass activity amongst all three reactors and the COD removal plateau was achieved faster compared to R1 and R3.

The study showed that the retrieved biomass from reactor R2, which was enriched with a well-acclimatized and diverse population of EABs like *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bactereodetes* (Fig. 3.4 and 3.5), exerted highest rate of substrate utilization. This may also be indicative of more favorable growth conditions for EABs inside the R2 reactor, compared to reactors R1 and R3. The heterotrophic activity of R3 was marginally higher than R1, which might have resulted due to the readily biodegradable organic source (acetate) provided in the synthetic feed. In all the reactors, the final VSS obtained was less than the initial VSS, which is a result of probable biomass loss during the decantation of reactors in between the cycles. The cumulative COD removal trend was found similar to Singh and Chakraborty (2021), which was performed for horizontal subsurface flow CW, however, they experienced a significant reduction in biomass activity at the end of the study due to low pH and prolonged exposure of biomass to acid mine wastewater laden with heavy metals.

3.3.6 Kinetic batch studies for fecal coliform removal

Constructed wetlands and microbial fuel cell technologies are associated with different mechanisms that result in the inactivation of pathogenic microorganisms present in the wastewater. In CW, the mechanisms that are responsible for pathogen removal are natural die-off, sedimentation, filtration, adsorption, etc. (Avelar et al., 2014; Khatiwada and Polpraser, 1999; Wu et al., 2016). The pathogen removal mechanism associated with MFCs is yet to be well understood, but some literature has connected the pathogen inactivation properties with bactericidal characteristics of the liquid catholyte, power generation process near the anode, and prevailing redox conditions suitable for external electron transfer in closed circuit mode

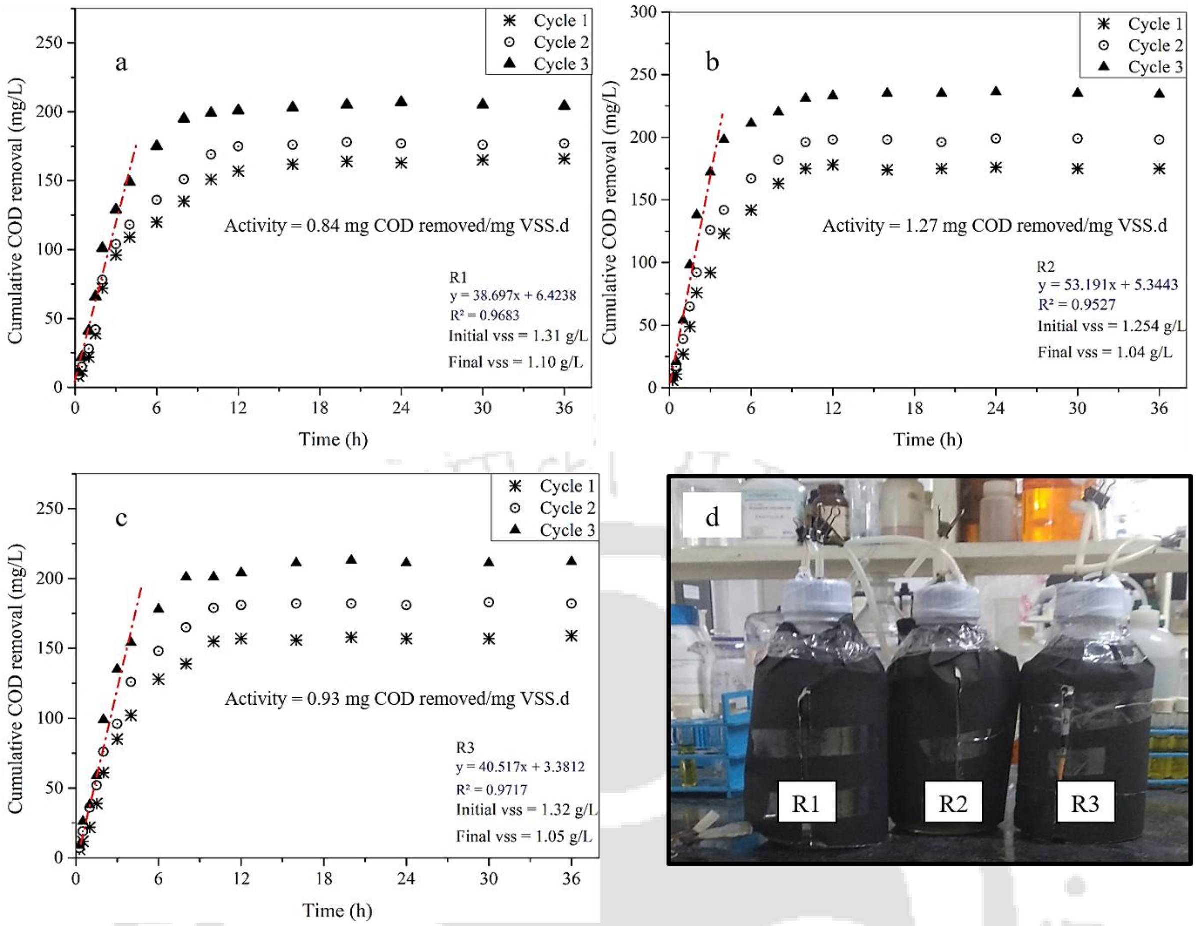

Fig. 3.6 Heterotrophic activity for cumulative COD removal in (a) R1, (b) R2, (c) R3 with time. (d) Photograph of the batch experimental setup used for the study

with a probability that active EABs near anode would outcompete exogenous microbial species in terms of organic uptake (Thorn et al., 2012; Cid et al., 2018). Other fundamental factors that can influence pathogen reduction in both systems are HRT, wastewater composition, pH, hydraulic regime, dissolved oxygen, solar intensity, and other seasonal factors (Jasper et al., 2013; Díaz et al., 2010; Headley et al., 2013). It is expected that all of these factors will exert a combined effect on FC removal in integrated CW-MFC.

When the reactors were operated in batch mode, the maximum OCV generated was 920 mV in reactor R2 and 475 mV in reactor R3, resulting in a maximum power density of 5.16 mW/m^2 for R2 and 1.91 mW/m^2 for R3. OCV output from 920 – 690 mV (up to 1st quartile) was considered as high EAB activity, from 690 – 356 mV (up to 1st quartile of max R3 OCV) was considered as medium EAB activity and below that, it was considered as low EAB activity.

Figure 3.7 illustrates the results obtained from different batch kinetic studies. The variation in OCV during the batch reactor operation in open and closed circuit mode has been illustrated in Figure 3.8. From Figure 3.7, the maximum natural die-off rate was observed in purged wastewater samples at a summer temperature of 31 ºC. During winter temperatures, the natural die-off rate for FCs in purged samples was higher than in unpurged samples. These results suggest that prevailing anaerobic conditions in the reactors contributed to FC inactivation, and seasonal temperature variation also affected FC removal (De Leén and Jenkins, 2002; Sydow et al., 2014). In batch reactor operation of R1, R2, and R3; maximum K value was obtained for reactor R2, due to combined effects of the anaerobic environment, EAB activity, and EAB-anode interaction; as all these factors are supportive for the EABs to thrive (Patel et al., 2021; Logan et al., 2019). Generally, two types of electron transfers take place in CW-MFC. One is microbial extracellular electron transfer to anode which results from organic decomposition by EABs and the other is electron transfer from anode to cathode via the external circuit. FC inactivation rate was found to be higher at sampling port S1 in reactor R3 compared to the S1 port of R1. This shows that the presence of an external electron acceptor as the anode was imparting positive effects.

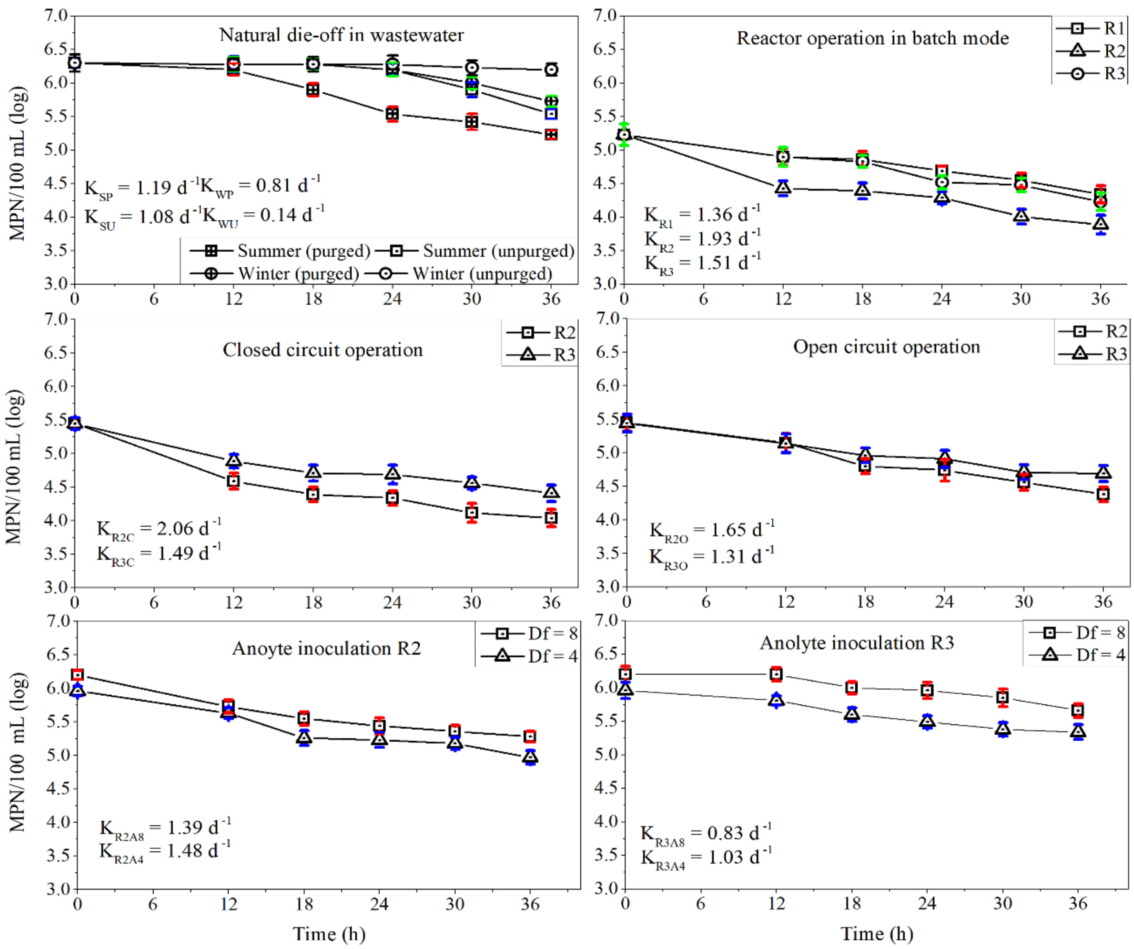

Fig 3.7 Kinetic analysis of FC count (as MPN/100mL) obtained from different modes of batch studies (abbreviations are explained in Table 3.1)

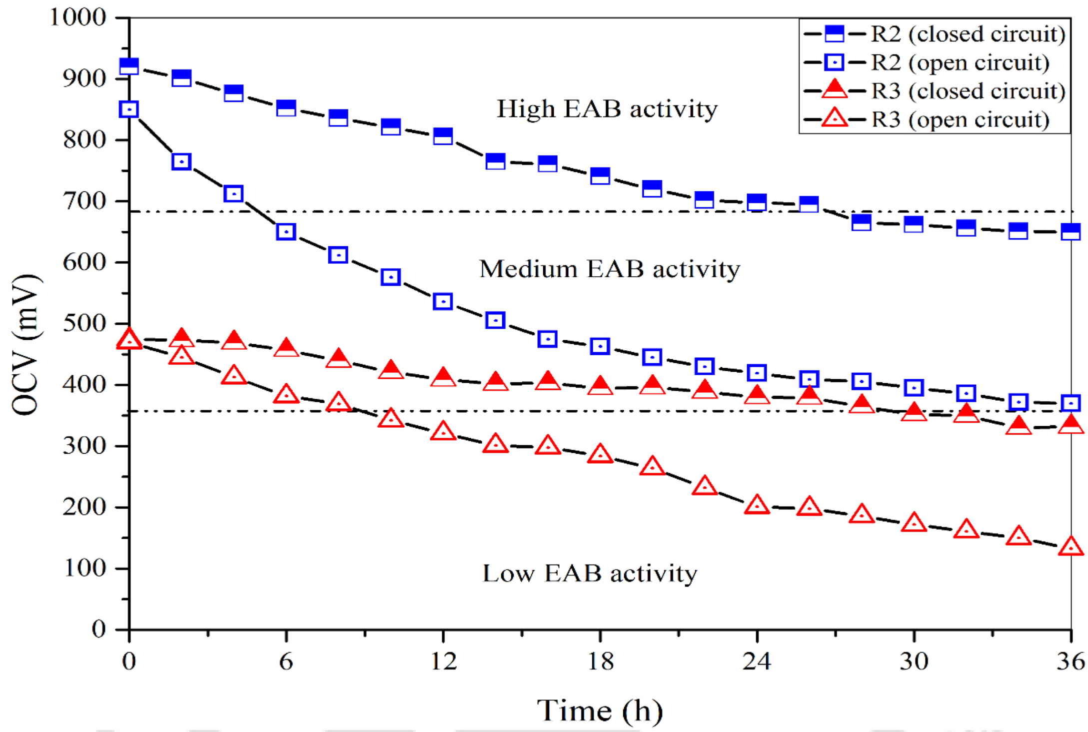

Fig. 3.8 OCV monitoring during batch reactor operation in open and closed circuit mode

For analyzing any probable effects of electron movement from the anode to the cathode, the R2 and R3 reactors were operated further in open circuit mode. During open circuit operation, a significant drop in source voltage was noticed. It was also observed that voltage drop during open circuit operation was comparatively less in planted reactor R2 than in unplanted reactor R3. A higher value was obtained for closed circuit operation of R2 and R3 compared to open circuit operation suggesting that generation of bioelectricity is positively correlated with FC removal and transferring of electrons to the cathode is an important factor in both planted and unplanted CW-MFC reactors.

A batch study with R2 anolyte inoculation in raw wastewater exerted a higher FC removal rate compared to the natural die-off rate with a dilution factor (D_f) of 4 and 8. However, in the case of inoculating raw wastewater with R3 anolyte, the die-off rate was reduced to less than the natural die-off. Anolyte inoculation from R2 led to higher FC removal as active EABs outcompete other heterotrophic microbes in the raw wastewater for food (Al Qarni et al., 2016). It is seen from OCV generation in Figure 3.8, that the EAB activity of R2 was higher compared to R3 during the operational period, so a similar amount of FC inactivation was not obtained after inoculating raw wastewater with R3 anolyte with a similar dilution factor. The obtained values of kinetic removal coefficients from different experimental conditions have been summarized in Table 3.1.

3.3.7 Organics and fecal coliform removal pathway in symbiotic CW-MFC mesocosm

It could be concluded that the integrated CW-MFC (R2) system was functioning as a symbiotic bioelectrochemical mesocosm where each element played a crucial role either actively or passively. The inherent symbiotic mechanisms are illustrated in Figure 3.9. Active interaction between the EABs and anode was important for getting a high rate of organic and fecal coliform removal. The current study was performed with domestic wastewater which had a high probability of containing refractory organics like detergents, pesticides, antibiotics, personal care products, disinfectants, etc., and reactor R2 has shown a higher COD removal performance compared other two reactors. In integrated CW-MFC systems, a higher redox gradient between the anode and the cathode results in better degradation of refractory organics (Yang et al., 2022). In the case of R3, the difference in the redox potential between the anode and the cathode was lower as compared to R2. This must have hampered the cathode

Table 3.1 Fecal coliform removal rate coefficients for different batch studies

Experimental condition	First-order removal rate coefficient (d^{-1})	Temperature (°C)
Natural die-off in purged wastewater during summer	K_{SP} = 1.19	31.0
Natural die-off in unpurged wastewater during summer	K_{SU} = 1.08	31.0
Natural die-off in purged wastewater during winter	K_{WP} = 0.81	23.4
Natural die-off in unpurged wastewater during winter	K_{WU} = 0.14	31.0
Batch mode reactor operation R1	K_{R1} = 1.36	28.3
Batch mode reactor operation R2 open circuit	K_{R2O} = 1.65	31.1
Batch mode reactor operation R2 closed circuit	K_{R2C} = 2.06	31.1
Batch mode reactor operation R3 open circuit	K_{R3O} = 1.31	31.5
Batch mode reactor operation R3 closed circuit	K_{R3C} = 1.49	31.5
Anolyte inoculation R2 (D_f = 4)	K_{R2A4} = 1.48	31.6
Anolyte inoculation R3 (D_f = 4)	K_{R3A4} = 1.03	31.5
Anolyte inoculation R2 (D_f = 8)	K_{R2A8} = 1.39	31.6
Anolyte inoculation R3 (D_f = 8)	K_{R3A8} = 0.83	31.5

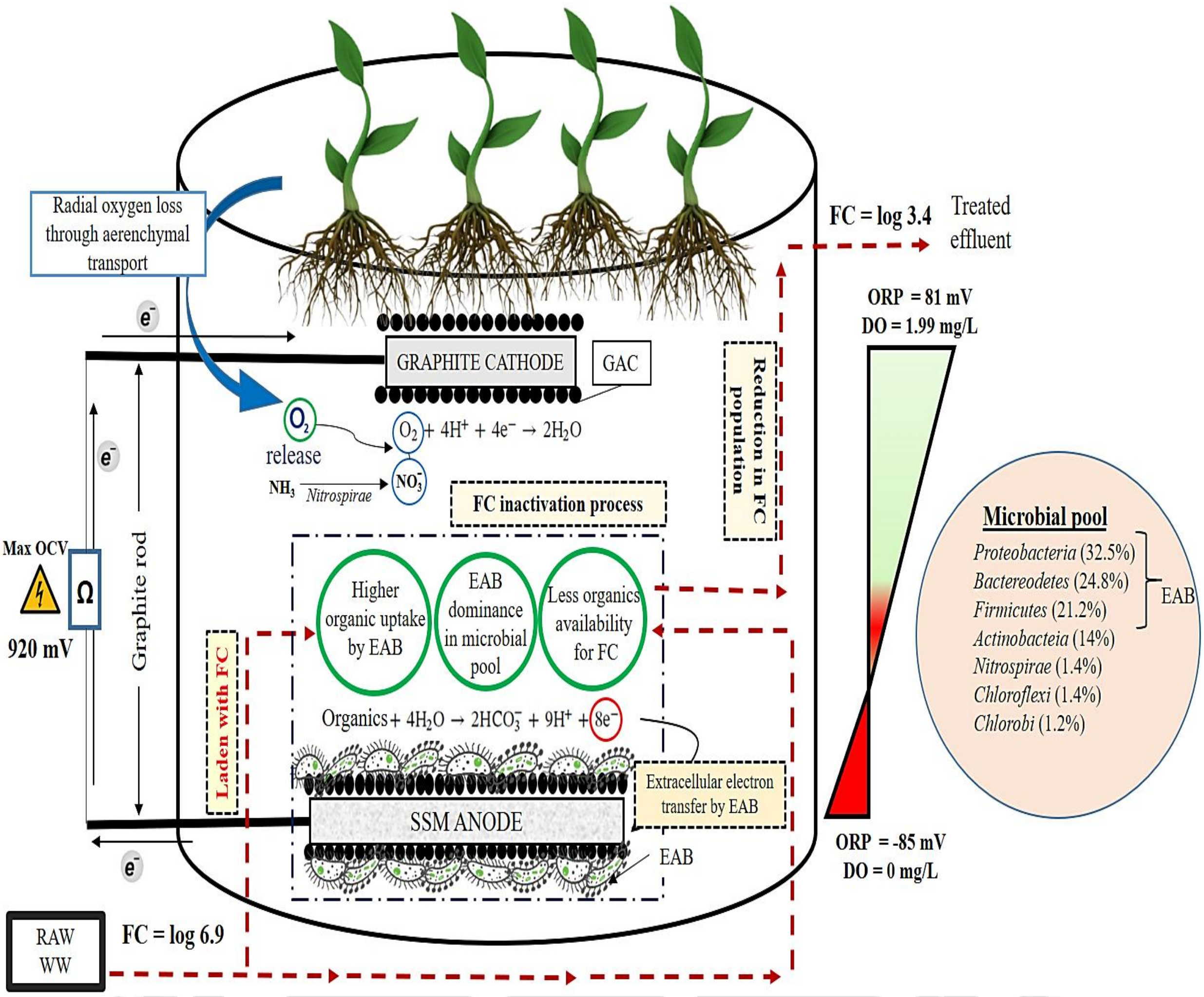

Fig. 3.9 Symbiotic bio-electrochemical pathway for organics and fecal coliform removal

cathode reaction and resulted due to a lack of DO availability and low ORP. This was the main reason for reactor R3 to render low COD removal efficiency compared to reactor R2. The plants played a positive role in recalcitrant organic degradation by facilitating the DO availability at the cathode and increasing the redox gradient in R2. Higher activity of EABs led to their dominance in the microbial pool which was also a key factor for higher organic uptake. Higher cathode potential led to a higher rate of electron transfer through the external circuit. The increased amount of electron flow resulted in higher open circuit voltage production in planted reactor R2. This electron transfer further accelerated the activity of EABs in the anodic zone. These phenomena resulted in less organic availability for other heterotrophic microbes (Non EABs), including the fecal coliforms present in the raw wastewater. These led to the maximum fecal coliform inactivation rate in R2 in closed-circuit operation conditions.

3.4. Conclusion and future scope

Taxonomic profiles of the microbial population in reactors R1 and R2 were more diverse compared to unplanted reactor R3 which can be attributed to the presence of plants. R2 was associated with an external electron acceptor (SSM anode) that helped EABs to thrive and subjugate other heterotrophic microbial species. During the activity study, R2 biomass showed the maximum specific substrate (acetate) utilization rate compared to R1 and R3 reactors. Assessment of the microbial population of the reactors showed dominance and diversity of EABs like *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* in the integrated CW-MFC reactor (R2). During the batch experiments conducted for the FC inactivation study, the highest removal rate coefficient was observed at the anode region of R2 during closed circuit operation. These results suggest a probable interconnection between the EAB activity in reactor R2 and FC inactivation when closed circuit operation ensured better electron transfer from the anode to the cathode. Integrated CW-MFC (R2), functions as a symbiotic mesocosm where EABs are a major factor for better organics and FC removal from raw domestic wastewater, compared to non-integrated standalone systems. The process of two-stage electron transfer (i) from EABs to the anode and (ii) from anode to cathode was found to be crucial for the desired level of FC removal. However, more studies in the future are required for in-depth investigation and understanding of the behavior and dominance of specific EAB species on indicator microorganisms like *Escherichia Coli* which is also capable of showing bioelectrical activity. Further investigations could be done to explore the capacity of pollutant remediation of integrated CW-MFC under varying operational conditions like hydraulic retention time, organic loading rate, etc. The contribution of the reactor media and the plant for organic removal by adsorption should also be investigated.

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Chapter 4

The Interrelation of Power Density and Pathogen Removal in Integrated CW-MFC Reactor under Varying Hydraulic Retention Time

This chapter is dedicated to the long-term operation of an integrated CW-MFC reactor under varying hydraulic retention times. The performance variation was monitored in terms of wastewater treatment and the bioelectrical response of the system. Special emphasis was given to investigate the interrelation of reactor power density and ambient temperature with the variation in effluent fecal coliform concentration under different retention times.

4.1 Introduction

Hydraulic retention time (HRT) is a major criterion for the design and operation of CW-MFC reactors. HRT can influence the microbial population associated with the electrodes inside the bioreactors which in turn will impact the power output and organic degradation efficiency (Sobieszuk et al., 2017). It is also responsible for altering the shear stress of the reactor fluid (wastewater in most cases) which governs biofilm formation and attachment on the electrode or media surface (Katsikogianni and Missirlis, 2010). It is considered one of the major operational parameters for CW-MFC that can affect bioelectricity production, power density, and coulombic efficiency (Wan et al., 2021). Significant effects of HRT on COD removal and bioelectricity extraction have been reported for pilot-scale MFCs. Sugioka et al. (2022), scaled up an MFC reactor up to 226L comprising 27 units and varied the HRT in a yearlong study. They have reported that the COD removal efficiency of the system increased from 31% – 58% when the HRT was varied from 9 h to 42 h. However, they observed a negligible difference in COD removal efficiency when HRT was increased from 24 h – 42 h. The influent COD range was 110 mg/L – 150 mg/L. Hiegemann et al. (2019) operated a submergible prototype MFC module (255 L volume) for the treatment of municipal wastewater. The HRT was varied at 43 h, 21 h, and 12 h. The maximum power density of the system was reduced from 50 mW/m^2 to 5 mW/m^2 due to the HRT reduction. However, the study did not find any significant correlation between temperature and bioelectrical performance of the reactor. The COD removal efficiencies during these three different HRT periods were 41%, 42%, and 28% respectively. Not much difference in COD removal was observed between the HRTs of 43 h and 21 h.

These findings prioritize more investigation on the optimization of the wastewater retention time for better reactor operation. As documented by some recent studies, CW-MFC reactors have exerted different levels of coliform removal while operated at different HRTs, which shows that the inactivation of coliform and pathogenic bacteria is largely dependent on the duration of their exposure to certain stress conditions inside CW-MFC reactors (Mittal et al., 2023; Colares et al., 2022). HRT also plays a crucial role in coliform removal in standalone MFC and constructed wetland systems (Khatiwada et al., 1999; Ieropoulos et al., 2017). However, increasing the HRT leads to an increase in the area requirement and the overall effective reactor volume which enhances the investment cost of the technology.

The current study explores the long-term operation and performance assessment of a lab-scale integrated CW-MFC reactor under varying HRT to observe the pollutant removal performance in terms of organics and coliforms and compare with standalone CW and unplanted CW-MFC reactors with similar layouts and operating conditions. The role of media in organic removal was evaluated by performing batch kinetic studies and the role of *Typha angustifolia* was also explored for any probable dual negative impact on the anode via radial oxygen loss.

4.2 Materials and methods

For this phase of the research, the reactor configurations, reactor designations, source of the wastewater, electrode arrangements, wetland macrophytes, etc. were kept identical as previously described in Chapter 2, Section 2.2.2. However, the hydraulic retention time (HRT) was varied by altering the peristaltic pump flow rate. The discharge rate of the peristaltic pumps was calculated (mL/min) using graduated measuring cylinders and the flow rates of the pumps were set accordingly as per the desired HRT and known reactor effective volume. In the current study, three reactors (R1, R2, and R3) were operated with an HRT of 36 h for an initial 325 days, then the HRT was reduced to 24 h for another 325 days, and finally, the HRT was further reduced to 18 h for 150 days. Reactor operation with 36 h, 24 h, and 18 h HRTs have been subsequently designated as Phase 1, Phase 2, and Phase 3 of the study. Some variation in the mean domestic wastewater characteristics was observed as compared to Chapter 2, due to an elongated period of sampling and analysis. Another major reason for the fluctuation in the wastewater characteristics may be the population increase. During Phase 1 of the research (as described in Chapter 2) the population of the Indian Institute of Technology Guwahati campus was less due to the enforcement of the Covid-19 lockdown in India, in the year 2021. The campus population gradually increased during Phase 2 and Phase 3 of the research after the withdrawal of the lockdown. This scenario probably affected the organic strength and other characteristics of the wastewater used for this research. The reactor setup used for this phase of the study has been illustrated in Figure 4.1.

4.2.1 Collection and characterization of domestic wastewater

Domestic wastewater was collected from the same point of source as mentioned previously in Chapter 2, Section 2.2.3. The characterization of the raw wastewater was done weekly once in terms of chemical oxygen demand (COD), 5-day biochemical oxygen demand (BOD_5), Dissolved oxygen (DO), pH, ammonia (NH_3-N), fecal coliform (FC), and total coliform (TC). The mean wastewater characteristics during the whole study period have been summarized in Table 4.1.

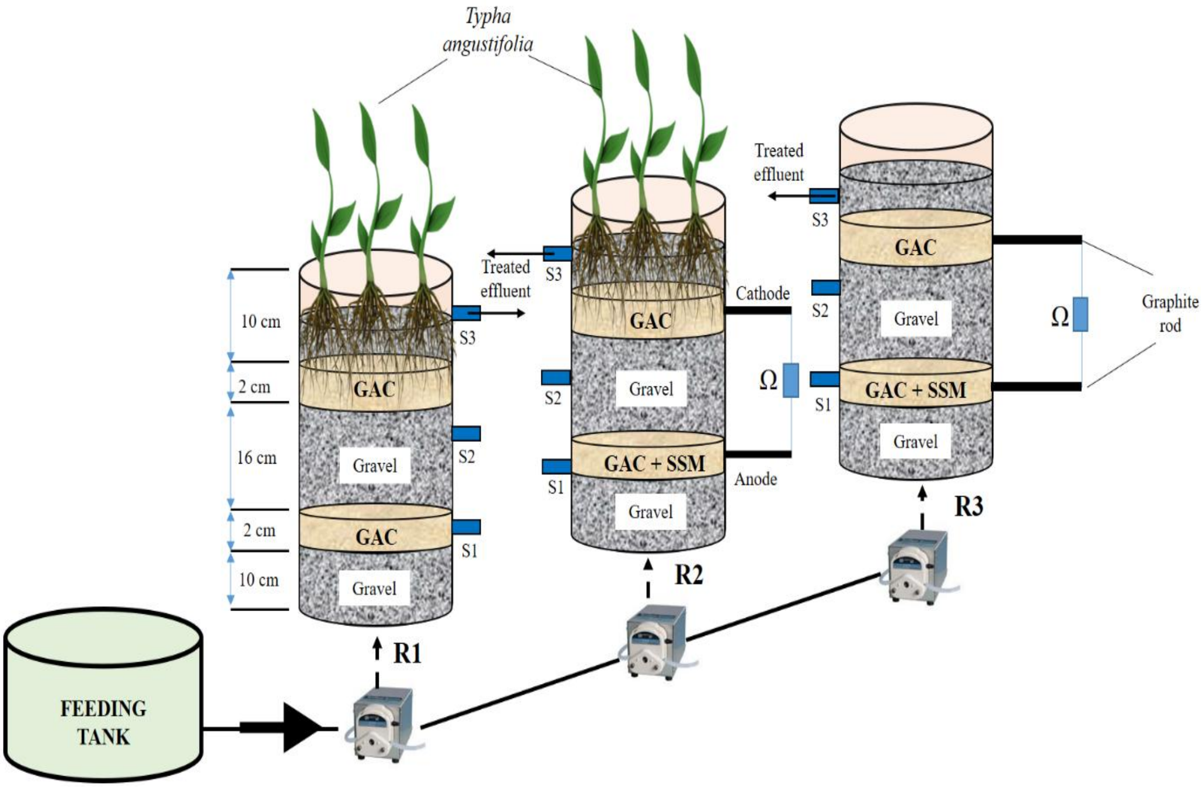

Fig. 4.1 Reactor configuration used in the current phase of the research

Table 4.1 Characterization of raw wastewater used in the current study

Parameter	Concentration
COD (mg/L)	192.85 ± 24.7
BOD_5 (mg/L)	112.15 ± 13.2
pH	7.15 ± 0.4
Total coliform (MPN/100 mL)	(9.54 ± 3.1) E+06
Fecal coliform (MPN/100 mL)	(7.76 ± 2.6) E+06
Dissolved oxygen	Not detected
Ammonia (mg/L)	20.94 ± 1.11

4.2.2 Analytical methods

Raw wastewater samples and the effluents from three reactors were periodically collected and analyzed during the complete study period. The study was performed in three phases having different hydraulic retention times (HRT). At different phases of the study, the performance of the three reactors was monitored in terms of wastewater treatment, and the bioelectricity generation in R2 and R3 reactors. Analysis of BOD_5, COD, pH, DO, ORP, total coliform (TC), and fecal coliform (FC) were carried out using the processes described in section 2.2.4 of Chapter 2. The ambient temperature was recorded with a digital thermometer associated with a metal probe (Thermomate ST-9283B). NH_3-N was measured by the Phenate method as described in the Standard Methods (APHA, 2017). All the samples were analyzed in triplicates to maintain the accuracy of the results and the lab experiments were performed adhered to the protocols prescribed in the Standard Methods.

Calculation of hydraulic retention time (HRT), COD and BOD_5 removal efficiencies, log removal of TC and FC, and the first-order rate coefficient for coliform inactivation were performed using equations 2.1, 2.4, 2.5, and 2.6 respectively as described in Chapter 2. For the Monitoring of the electrical performance of reactors R2 and R3, current (I), power (P), power density (PD), and current density (CD) were measured using equations 2.7, 2.8, 2.9, and 2.10 respectively as described in Chapter 2.

4.2.3 Batch experiment studies

Batch studies were carried out to estimate the contribution of media and the attached biomass for COD removal after the completion of Phase 3 of the study. In the first batch study, the amount of biomass associated with the media was investigated by obtaining gravel, macrophyte root, and GAC from all the reactors. The specific volatile suspended solids (VSS) study was performed by extracting 10 g, 20 g, and 30 g of gravel; 5 g, 7.5 g, and 10 g of GAC; 2.5 g, 5 g, and 7.5 g of macrophyte root from the reactors. Roots were obtained only from reactor R1 and reactor R2 since reactor R3 was unplanted. The media was kept in a 250 mL conical flask with 100 mL distilled water and was kept in the horizontal shaker for 6 h at 150 rpm. After shaking, the liquid was decanted immediately from the flask and was analyzed for VSS. The results are reported as mg VSS/g media.

The second batch kinetic study was performed to analyze the mass COD removal rate (mg COD removed g media^{-1} d^{-1}) exerted by different media. 30 g gravel, 20 g GAC, and 5 g *Typha* root were put into a 250 mL conical flask with 100 mL of purged synthetic distilled water with an initial COD of 250 mg/L (sodium acetate was used as the sole carbon source). The batch study was continued for 36 h (similar to reactor HRT in Phase 1), in a horizontal shaker at 50 rpm and the COD remaining was assessed at an interval of 0 h, 8 h, 16 h, 24 h, and 36 h.

4.2.4 Statistical data analysis

All statistical data analysis was performed using MS Excel software (version 16). One-way ANOVA analysis was applied for the results of different reactors to assess the significance of the difference in the outcome with a p-value < 0.05. Multilinear regression modelling was performed using the MS Excel data analysis tool package with a 95% confidence limit and the predictive plots with actual values was generated. Residual plots corresponding to each predictive regression model were developed to check the applicability of linear regression for the current dataset.

4.3 Results and discussion

In the current chapter, the HRT was not increased beyond 36 h since wastewater treatment performance in Phase 1 was satisfactory in terms of organics removal and coliform inactivation. If the HRT of the integrated CW-MFC reactor is increased beyond a period of 36 hours, it may result in a larger area footprint. This might become a difficult hurdle to overcome toward the economic application of this reactor at the community level in rural and suburban sectors.

4.3.1 Effect of HRT on organic and coliform removal

The organic removal performance of the reactors during Phase 1, Phase 2, and Phase 3 was determined by the COD and BOD_5 removal efficiencies. Reduced HRT showed a negative impact on organic removal performances as all the reactors have shown a spike in effluent organic content in Phase 2 and Phase 3, as compared to Phase 1. This is justified as reduced HRT leads to less contact time available for the heterotrophic microorganisms to utilize and decompose the dissolved organic matter present in the wastewater (Fang et al., 2017; Li et al., 2013). However, in Phase 2 and Phase 3, the effluent COD in reactors R1 and R3 was higher compared to Reactor R2. An increase in the organic content of raw wastewater in Phase 2 and Phase 3, compared to Phase 1 was observed. Figures 4.2 (a) and 4.2 (b) illustrate the variation in effluent BOD_5 and COD for different phases of the study. The COD concentration in the raw domestic wastewater was observed to be in the range of 150 mg/L to 250 mg/L. Better COD removal in Reactor R2 compared to reactors R1 and R3 shows the ability of the planted CW-MFC system to decompose organic matter at a faster rate compared to CW and unplanted CW-MFC. One-way ANOVA analysis was performed to inspect significant differences ($p<0.05$) among the effluent COD and BOD_5 concentrations of the reactors during the study. A significant difference was observed in the effluent COD of reactor R2 compared to reactors R1 and R3 during all the phases (see Appendix C, Table C1 – Table C6).

COD removal in bioelectrochemical systems is largely dependent on the process of electron transfer at the anode by the electroactive bacteria (EAB) and the subsequent flow of electrons from the anode to the cathode (Tang et al., 2019). Higher COD removal could be a result of increased electrogenic microbial activity near the anode and a higher rate of electron flow via the external circuit in reactor R2. This is facilitated by the macrophytes by increasing the cathode redox potential through root-level oxygen release (Yang et al., 2021; Yang et al., 2020; Zhang et al., 2011). Not much difference in effluent BOD_5 of the reactors was observed during Phase 1 and Phase 2. One major reason for this could be the low organic strength of the raw domestic wastewater which showed BOD_5 concentration in the range of 91 mg/L to 129 mg/L during the complete study period. Only in Phase 3 (HRT 18 h), reactor R2 has shown a significant difference in effluent BOD_5 as compared to R1 and R3 (see Appendix C, Table C7 – C12). This suggests, that an integrated CW-MFC system could be a suitable option for the treatment of low-strength biodegradable wastewater when the operational HRT is as low as 18 h. The mean BOD_5 and COD removal efficiencies of the reactors are summarised in Table 4.2.

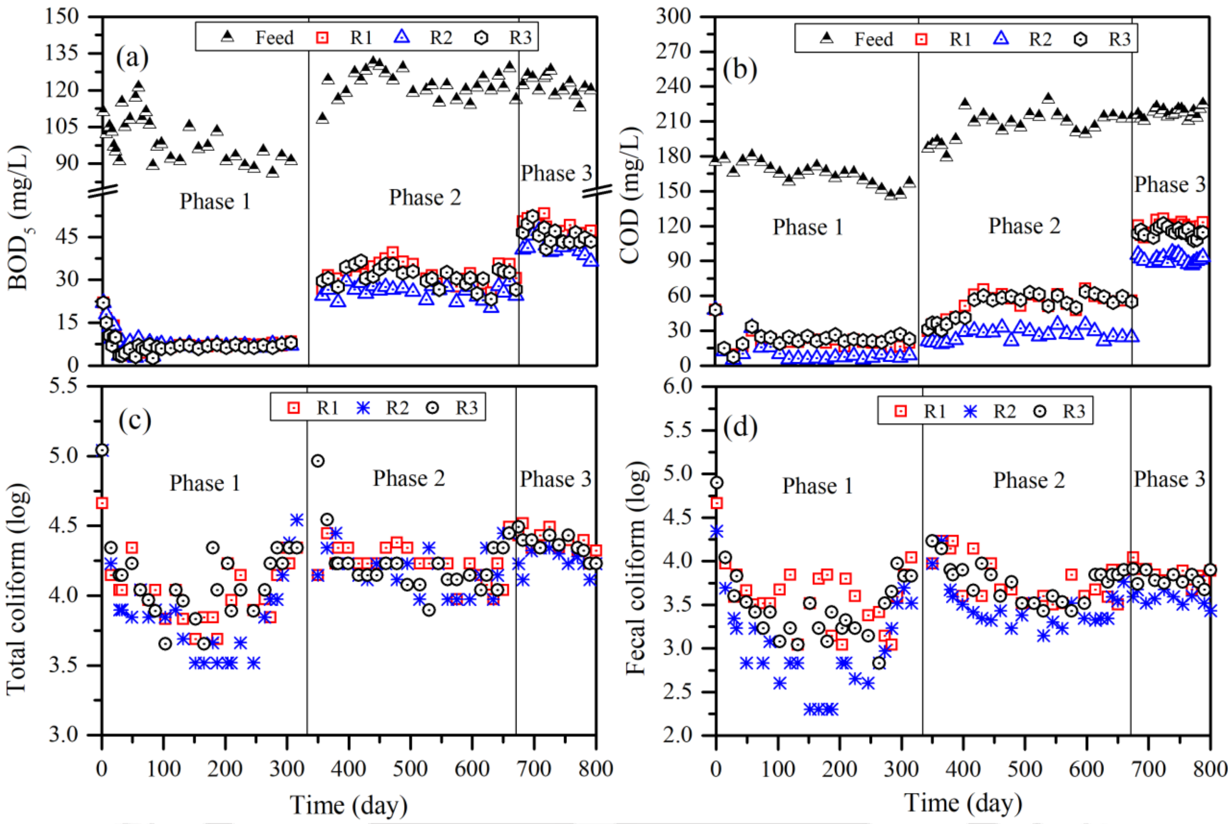

Fig. 4.2 Variation in (a) effluent BOD_5, (b) effluent COD, (c) effluent TC, and (d) effluent FC concentration in different reactor effluents during the study. The data reported for Phase 1 in the figure was obtained from Chapter 2.

The variation in effluent total coliform (TC) and fecal coliform (FC) concentration (represented in log scale) for reactors R1, R2, and R3 at different phases have been illustrated in Figures 4.2 (c) and 4.2 (d). Similar to organic removal, the coliform inactivation in all reactors was considerably reduced in Phase 2 and Phase 3 due to a reduction in HRT. During Phase 1, R2 showed the lowest effluent FC and TC concentration. Comparative FC concentration in R3 effluent was found to be lower than R1 which may be attributed to the activity of the electroactive bacteria in the presence of an anode as the external electron acceptor (Colares et al., 2021; Ieropoulos et al., 2019). In Phase 2 and Phase 3, with reduced HRT of 24 h and 18 h, Reactor R2 was capable of removing FC from raw wastewater comparatively better than R1 and R3, but the difference was reduced. The performance of Reactor R2 in TC removal was marginally better in Phase 2 and Phase 3, as compared to reactors R1 and R3. The mean coliform removal efficiencies of the reactors in different phases are summarized in Table 4.2. The results obtained in the current study were similar to that have been reported by Colares et al. (2021) and Afzal et al. (2019). The findings of these studies have emphasized that the presence of macrophytes led to a significant increase in coliform bacteria inactivation for integrated planted CW-MFC reactors while treating domestic wastewater. From the effluent quality assessment of the whole study, it can be affirmed that optimum HRT is an essential factor for obtaining better coliform removal in planted CW-MFC systems. The effluent produced by the integrated CW-MFC reactor (R2) during Phase1, could be classified as class E (irrigation, industrial cooling, and controlled waste disposal) as per the designated best use of water prescribed by CPCB in 2009, and the FC standard prescribed by the Ministry of Urban Development India in 2004 (Seth, 2016). For Phase 2 and Phase 3, the R2 effluent may be classified as Below E. The CPCB standard for the designated best use of water is provided in Appendix C, Table C14.

4.3.2 Effect of hydraulic retention time on bioelectricity production

The influence of hydraulic retention time on bioenergy production in reactors R2 and R3 were monitored throughout the study period. Figure 4.3 (a) shows the variation in the open circuit voltage (OCV) generation by different reactors in Phase 1, Phase 2, and Phase 3. During Phase 1, both R2 and R3 took around 100 days to reach maximum voltage output post-reactor commissioning. In Phase 1, the maximum open circuit voltage (OCV) exerted by reactors R2 and R3, were 920 mV and 487 mV. In Phase 2, the HRT was reduced to 24 h which resulted

Table 4.2 Organics, TC, and FC removal in different phases by reactors R1, R2, and R3

Parameters	**HRT (h)**	**Removal Efficiency**		
		R1	**R2**	**R3**
BOD_5 (mg/L)	36	91.79±3.58 %	92.71±3.30 %	92.09±3.64 %
	24	73.71±2.16 %	75.89±3.01 %	74.37±2.24 %
	18	60.56±2.36 %	65.61±2.47 %	62.52±2.50 %
COD (mg/L)	36	87.21±4.56 %	93.13±4.83 %	86.31±4.37 %
	24	74.58±3.74 %	87.15±1.85 %	75.48±3.93 %
	18	45.01±8.11 %	57.73±8.88 %	47.07±7.05 %
Total Coliform (MPN/100 mL)	36	log 3.03±0.16	log 3.18±0.21	log 2.96±0.13
	24	log 2.74±0.14	log 2.78±0.14	log 2.75±0.17
	18	log 2.52±0.16	log 2.64±0.15	log 2.54±0.11
Fecal coliform (MPN/100 mL)	36	log 3.34±0.11	log 3.95±0.17	log 3.45±0.12
	24	log 3.09±0.12	log 3.39±0.14	log 3.11±0.14
	18	log 3.02±0.16	log 3.26±0.13	log 3.07±0.19

in a significant voltage drop for both the reactors though R2 showed higher OCV compared to R3. Another considerable drop in the output OCV was noticed when HRT was further lowered to 18 h after 650 days of reactor operation. For reactor R2, the drop-off in maximum OCV output during Phase 2 and Phase 3 were 21.55% and 51.58% respectively compared to the maximum OCV observed in Phase 1. Similarly, for reactor R3; the maximum OCV output was reduced by 32.85% and 56.46% in Phase 2 and Phase 3, as compared to Phase 1.

Figure 4.3 (a) shows that, during the start of every phase, R2 and R3, have taken some amount of time to produce steady output voltage after a rapid initial drop in OCV. This might be due to the EABs in the reactors that took their time to acclimatize to the rapidly modified operational conditions during the phase alterations. During the study, an overall downturn was observed in the output OCV of the reactors. The major reason for bioelectricity production in an MFC or an integrated CW-MFC system is the presence of electroactive bacteria (EAB) and their activity and concentration in the vicinity of the anode (Ramírez-Vargas et al., 2019). A reduced HRT increases the wastewater flow rate which could alter the mixing condition of the flow regime inside the reactors and affect microbial activity by distorting the biofilm attached to the electrode surface. On the contrary, increased HRT enhances the substrate residence time within the reactor and increases the EAB contact time leading to more amount of electron production via organic decomposition and subsequent electron transfer to the anode resulting in enhanced voltage production (Sobieszuk et al., 2017; Sharma and Li, 2010). The results obtained in the current study were similar in trend with literature when an increased HRT has led to higher bioelectricity production in MFCs due to EAB activity (Ye et al., 2020; Srikanth et al., 2016; Rahimnejad et al., 2011).

In a very recent study, Yang et al. (2022), operated one integrated CW-MFC reactor with varying HRT and observed similar results in terms of voltage generation. They have reported an output voltage of 460 mV with 48 h HRT but the voltage was dropped to 388 mV and 215 mV when HRT was reduced to 24 h and 12 h respectively; with a feed COD of 200 mg/L. There might be an apparent conclusion from the current study that increased HRT leads to higher OCV. However, prolonged HRT could negatively impact bioelectricity production and reduce the output voltage by destroying the anaerobic conditions at the anode (Saeed and Sun, 2012). For each phase, polarization plots were obtained for R2 and R3 reactors by connecting different external resistances varying from 1Ω to 1 MΩ. As, the study was performed with raw domestic wastewater, keeping in mind the probable daily fluctuations in organic content; the polarization study was done after the reactors showed stabilized OCV production for at least 60 days in each phase.

Figures 4.3 (b) and 4.3 (c) illustrate the polarization plots obtained for reactors R2 and R3 in different phases of the study. The power density (PD) and current density (CD) were reported per unit projected area of the SSM anode. During Phase 1, the maximum PD exerted by Reactor R2 and Reactor R3 was 5.5 mW/m^2 and 1.32 mW/m^2 respectively (as mentioned in Chapter 2). In Phase 2, the maximum PD observed from R2 and R3 reactors was reduced to 2.96 mW/m^2 and 0.44 mW/m^2. PD was further reduced to 0.74 mW/m^2 and 0.18 mW/m^2, for reactors R2 and R3 in Phase 3 of the study. A decrease in PD with reduced HRT was previously observed for MFC reactors by Sorgato et al. (2023) and Subha et al. (2019). This phenomenon could be explained by the increased organic loading rate (OLR) experienced by the reactors due to a reduction in HRT. High OLR with reduced HRT may hamper the electron transfer to

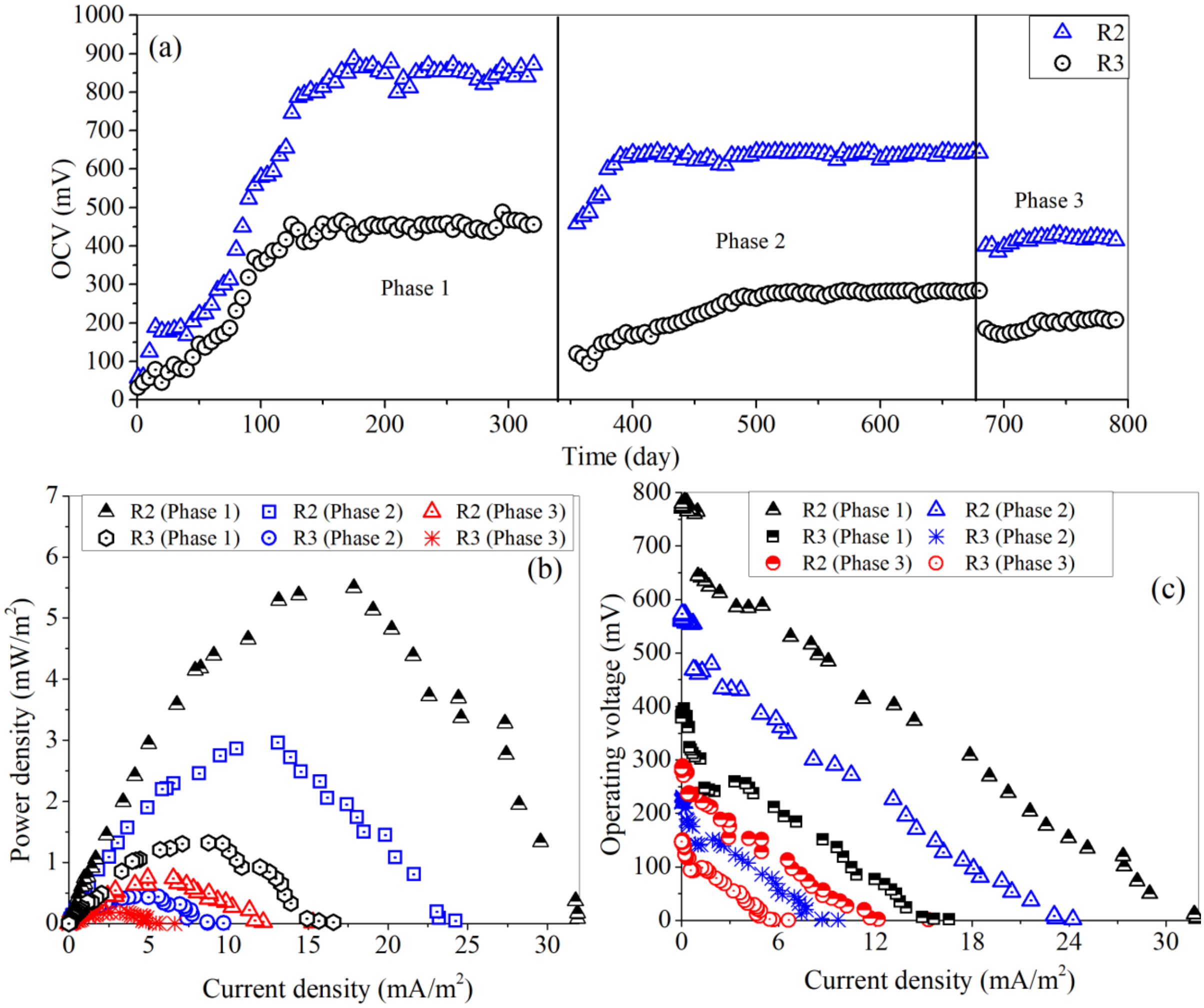

Fig. 4.3 Variation in OCV and corresponding polarization plots for R2 and R3 during different phases of the study

the anode and proton transfer to the cathode which reduces the organic decomposition capabilities of the EABs leading to the production of low power densities (Yang et al., 2022). Table 4.3 summarizes the maximum PD, CD, OCV, and the OLR of reactors R2 and R3; as was observed during different phases of the study.

4.3.3 Role of media as support matrix and involvement in organic removal

Media substrate, also known as the media matrix or support matrix of the reactors is a fundamental part of any integrated CW-MFC system. Media play a very important role in pollutant removal by various physicochemical mechanisms and also provide the required surface area in a bioreactor for the microbes to attach and grow, thereby influencing biological organic decomposition (Jingyu et al., 2020). The current study used gravel as the filler media and granular activated carbon (GAC) as the support media at the anode and cathode. Other than gravel and GAC, the roots of the macrophytes were also present in reactors R1 and R2, which provided a sufficient surface area for the attachment of bacteria and adsorption of organic pollutants (Yang et al., 2020). The specific VSS values obtained per unit mass of the reactor media (GAC, gravel, and root) are summarized in Table 4.4. Amongst all the reactors, the maximum specific VSS was obtained from GAC. The highest VSS was obtained per unit mass of GAC due to its high specific Langmuir surface area (3.74 m^2/g). The result of the specific

Table 4.3 Variation in OLR and other electrical parameters during the study in R2 and R3

Parameters		Reactor R2	Reactor R3
OLR ($gCOD/m^2.d$)	HRT 36 h (Phase 1)	16.24	16.24
	HRT 24 h (Phase 2)	30.23	30.23
	HRT 18 h (Phase 3)	41.27	41.27
Maximum OCV (mV)	HRT 36 h (Phase 1)	925	487
	HRT 24 h (Phase 2)	695	324
	HRT 18 h (Phase 3)	429	212
Maximum PD (mW/m^2)	HRT 36 h (Phase 1)	5.50	1.32
	HRT 24 h (Phase 2)	2.96	0.44
	HRT 18 h (Phase 3)	0.74	0.18
Maximum CD (mA/m^2)	HRT 36 h (Phase 1)	31.88	16.58
	HRT 24 h (Phase 2)	24.23	9.69
	HRT 18 h (Phase 3)	15.31	6.63

Table 4.4 Specific VSS values obtained from different reactor media

Reactor	VSS obtained per unit mass of media (mg VSS/g media)		
	GAC	*Typha* Root	Gravel
R1	469.38±77.79	187.13±27.31	31.12±2.84
R2	536.74±77.22	232.12±34.47	29.31±2.85
R3	487.26±76.13	N.A.	30.59±3.32

surface area analysis of GAC is provided in Appendix C (Fig. C1). Gravel exerted the minimum specific VSS value due to its smooth surface structure.

Figure 4.4 shows the results of the batch kinetic study performed to determine the mass COD removal rate by different media. The samples were analyzed in triplicates and a total of three trials were executed for each set of samples to ensure repeatability and data accuracy. Another set of studies was done with autoclaved media to avoid microbial COD degradation and to assess the role of media only. It could be observed from Figure 4.4, that autoclaved media rendered a very low mass removal rate compared to non-autoclaved media suggesting bacterial decomposition is majorly responsible for soluble organic removal during reactor operation. Some amount of COD removal was obtained from the autoclaved media probably due to surface adsorption, but GAC and *Typha* root did not show a high mass removal rate despite having more surface area as compared to gravel. The mass removal rates of different media (autoclaved and non-autoclaved) are illustrated in Figure 4.5, The probable reason behind this is the high microbial attachment on GAC surface and roots which left very little free surface area on the respective media. The data obtained from these batch studies emphasize that the main role of the media (gravel and *Typha* roots) was to provide a surface for the bacteria to attach and grow inside the reactor. Due to the high specific surface area of GAC, some organic removal is expected but the batch study results affirm that the effect of adsorption is negligible in the long-term reactor operation once microbial biofilm is fully grown on the media surface.

4.3.4 Investigation of the dual impact of *Typha angustifolia*

Matured *Typha angustifolia* plants were acquired from a local marshland in the Indian Institute of Technology Guwahati (IIT Guwahati) campus and were used for the batch study to separately analyze the contribution of macrophytes for BOD_5 and COD removal. Three plastic

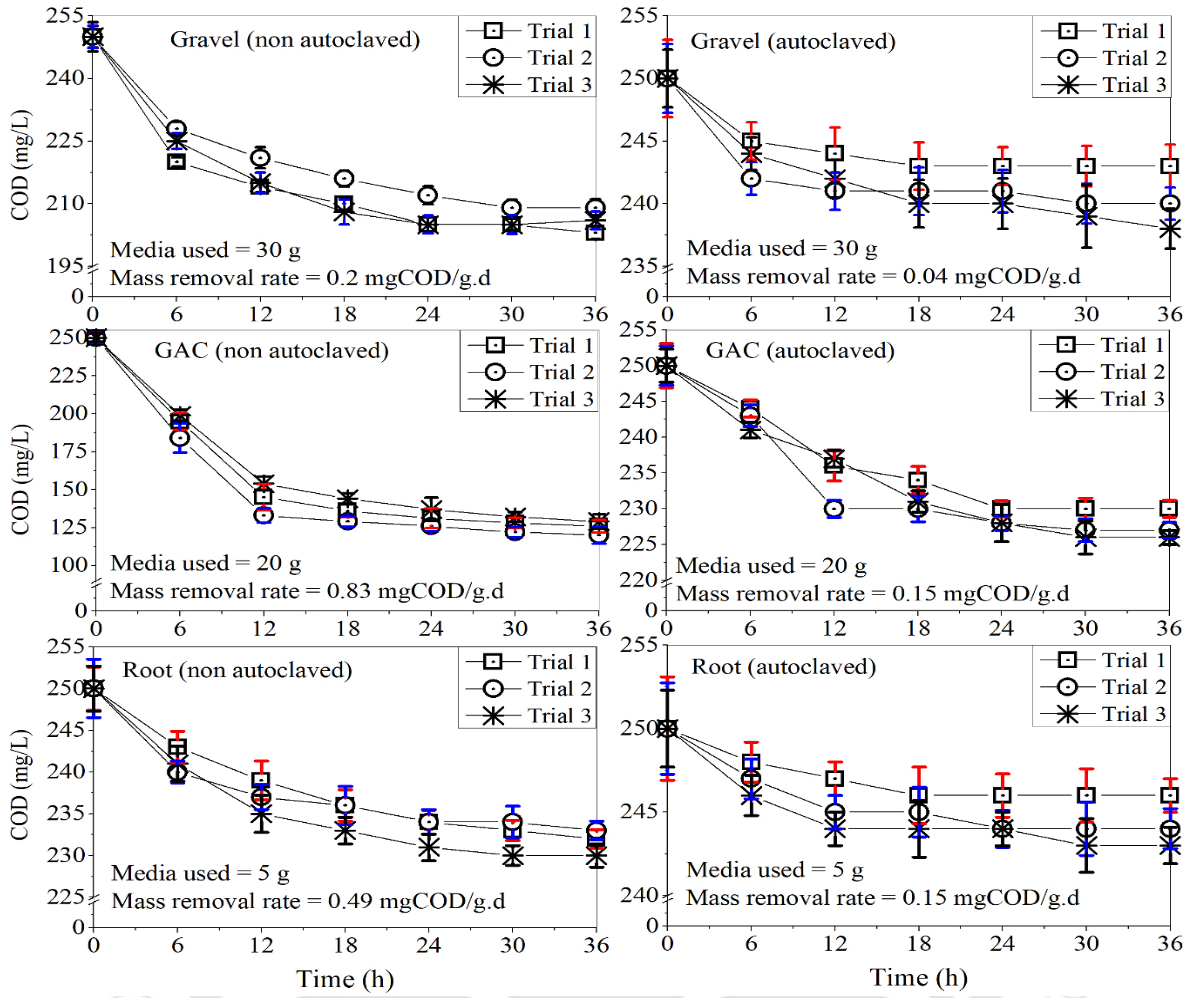

Fig. 4.4 Batch study with autoclaved and non-autoclaved media for organic removal

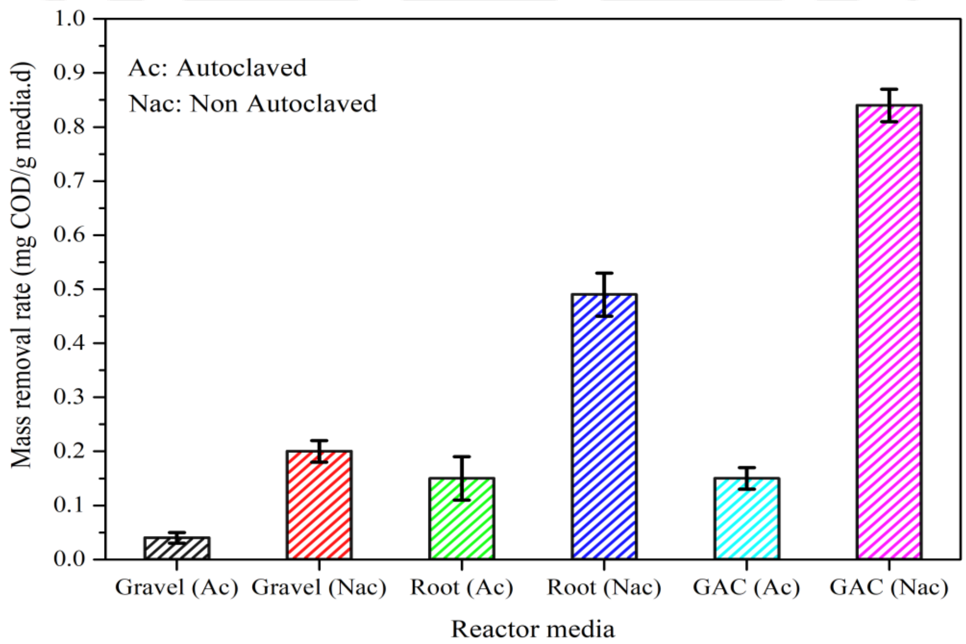

Fig. 4.5 Comparison of COD mass removal rate obtained from autoclaved and non-autoclaved media in the current study

pots namely, Pot 1, Pot 2, and Pot 3 were filled with 500 mL of raw domestic wastewater having initial COD 205.33±9.07 mg/L and BOD_5 119.67±5.86 mg/L. Pot 1 was kept as a control without plant. Pot 2 was kept with two plants and Pot 3 was kept with one single plant.

Plant roots were thoroughly washed with tap water multiple times followed by deionized water to remove microbes attached to the roots to avoid microbial degradation of the organics that were attached to the plants. COD and BOD_5 were measured at T = 0 h and T = 36 h. The photograph of the experimental setup is provided in Appendix C (Fig. C2). The mean COD removal efficiency for Pot 1 (control), Pot 2 (dual plant), and Pot 3 (single plant) were 46.59%, 47.89%, and 46.75% respectively. The mean BOD_5 removal efficiencies obtained for unplanted, single-plant, and dual-plant pots were observed as 38.39%, 36.53%, and 38.03%.

Results suggest that organic removal obtained in all the pots was primarily due to the metabolic activity of the microbes that were abundant in raw wastewater. The results of the study have been summarised in Table C13 of Appendix C. The batch study with *Typha angustifolia* disclosed that the plants are unable to contribute in terms of COD or BOD_5 removal from raw wastewater without microbial attachment to the roots. Though some literature has reported that macrophytes release organic root exudates, it showed little impact on the COD or BOD_5 content in the current batch study probably due to less number of plants used per pot and the short duration of the study (36 h) (Jensen et al., 1982).

Macrophytes play two major roles in CW-MFC reactors: (i) Increase the redox potential near the cathode through radial oxygen loss by roots that result in better EAB activity and bioelectricity production, and (ii) Provide sufficient surface area to the bacteria and increase attached microbial population and diversity near the rhizosphere. During the current study, Reactor R2 (planted) continuously showed better wastewater treatment performance and voltage generation compared to unplanted R3. These results are akin to multiple previous pieces of research (Wen et al., 2020; Zhou et al., 2018, Oon et al., 2017). Most literature has largely concentrated on the positive aspects of wetland plants in CW-MFC reactors. However, in the case of long-term reactor operation, roots may grow and reach the bottom part near the anode and hamper anode-EAB activity by releasing oxygen at a minute level (Yang et al., 2020). To investigate the effects of radial oxygen loss from the *Typha* roots, the dissolved oxygen (DO) concentration and the redox potential (ORP) were measured at sampling ports S1, S2, and S3 in all the reactors. The mean DO and ORP values obtained from different sampling ports throughout the complete study have been illustrated in Figure 4.6. For further investigation of the possible dual role of macrophytes, plants from reactor R1 were uprooted

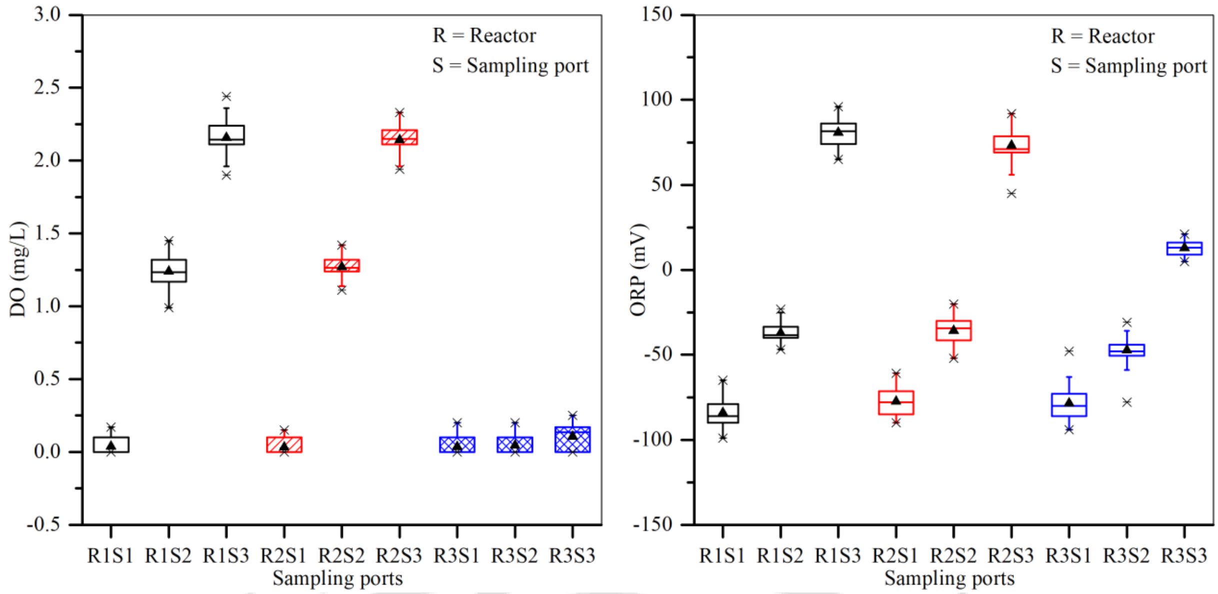

Figure 4.6 Spatial variation of DO and ORP in the reactors during the complete study period

and the root length was measured after the completion of Phase 3, while R2 was kept intact. At the end of Phase 3, the number of matured plants was similar in reactors R1 and R2 (7 plants in each reactor) with average plant heights of 193.5 cm and 190.7 cm respectively. Both of the reactors have shown similar DO and ORP variation throughout the study under identical operating conditions at different phases. Detailed images of the harvested plants with the root sections have been provided in Appendix C (Fig. C3 and C4).

During the macrophyte harvesting, the presence of roots was not observed after a depth of 25 cm from the top surface of Reactor R1. The depth of the anode in reactors R2 and R3 was 30 cm from the top, thus the observed DO near the anode (sampling port S1) was zero even in the planted reactors. For further evidence and to obtain a better insight into the rhizome network, roots with undamaged tips were obtained from planted reactors R1 and R2, and the FESEM images of root and root cross sections were taken. Figure 4.7 shows well-developed aerenchyma tissue in the roots obtained from reactors R1 and R2. These mature aerenchyma tissues are crucial for oxygen transportation from shoots to roots and further to the rhizosphere (Oon et al., 2016). From the results, it is concluded that *Typha angustifolia* played a positive role in the enhancement of bioelectricity production and pollutant removal in integrated CW-MFC systems. The roots did not reach the anode, thereby maintaining favorable anaerobic conditions for the EABs to grow and produce bioelectricity. However, in Reactor R3, the absence of plants led to the reduced potential difference between the cathode and anode which

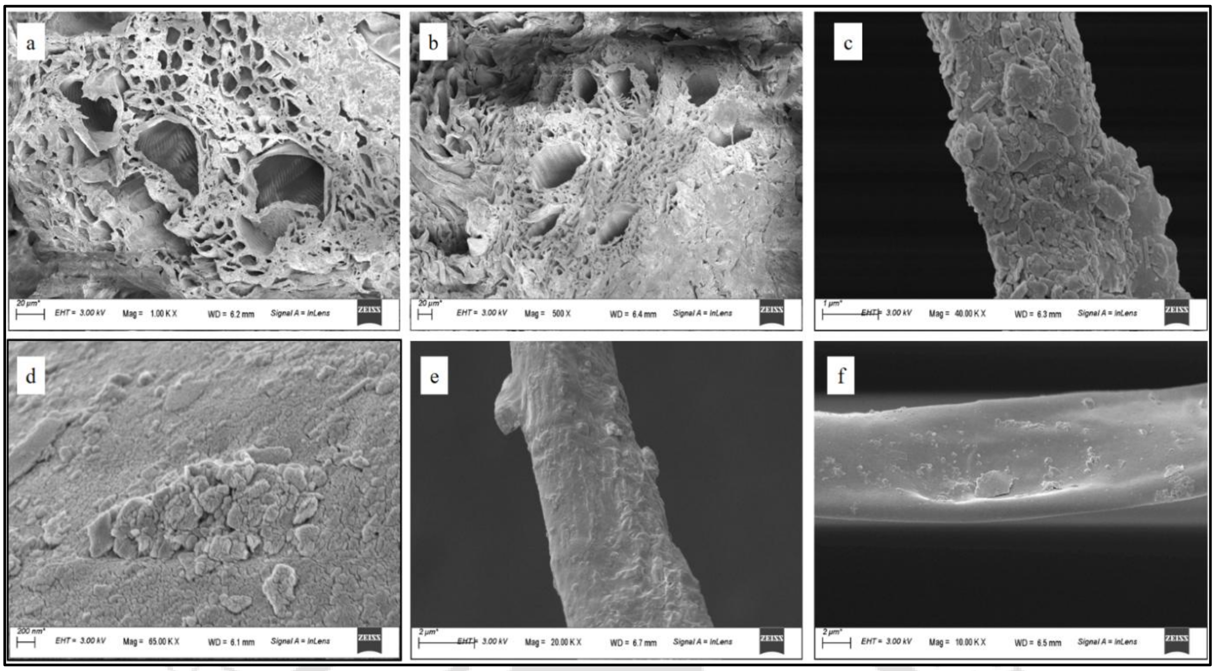

Figure 4.7 FESEM images of root cross section obtained from (a) R1 and (b) R2 showing mature aerenchyma tissue; (c) microbial abundance on the tertiary root of R1, (d) clustered attachment on the tertiary root of R2, (e) and (f) reduced amount of attached materials on the roots after thorough washing

Led to a less amount of electron flow via the external circuit which demonstrated low bioelectricity generation as compared to Reactor R2.

4.4 Conclusion

Some major insights could be portrayed from the outcomes of the current study which could potentially be used for field application and long-term operation of integrated CW-MFC reactors. The steady state of bioelectricity production by Reactor R2 for 800 days in different phases under different operating conditions answers the acclaimed doubts regarding bioreactor durability. GAC was used near the electrode regions (cathode and anode) to enhance the microbial attachment and the maximum amount of specific volatile suspended solids was obtained from it. Plant roots were also capable of providing a considerable amount of microbial attachment. The amount of specific volatile suspended solids retrieved from the *Typha* roots was much higher than the gravel media but was less than that of GAC. Comparative batch studies with autoclaved media revealed that in the long run, the individual contribution of GAC, plant rhizome network, and gravel media towards organics removal was negligible without the active biomass attached to the surface. HRT evolved as a major factor governing the wastewater treatment performance and electrical performance on the integrated CW-MFC system. Reactor R2 performed best with 36 h HRT. Periodic reduction in the HRT resulted in reduced coliform removal efficiency of the reactors along with depreciated bioelectricity production. *Typha angustifolia* played crucial roles by providing surface area for biomass attachment and by increasing the cathode redox potential of Reactor R2, due to radial oxygen loss via matured root aerenchyma tissue. Further exploration of the reactor interior environment revealed that macrophyte roots did not reach the anode vicinity despite a long-term reactor operation of 800 days. This negates any chance of perturbation in the anaerobic environment near the anode. The omittance of the proton exchange membrane and replacing it with a compact non-conductive gravel layer helped the purpose of reactor economy and durability. However, it is of utmost necessity to investigate the economic benefits of the integrated CW-MFC system compared to other reactors used in this research to showcase the potential of sustainable wastewater management and valorization.

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Chapter 5

Economic Aspects of Integrated CW-MFC System for Domestic Wastewater Treatment: A Cost-Benefit Approach

The current chapter endeavored to execute a comparative performance analysis of reactors R1, R2, and R3 to estimate the potential economic benefits offered by the individual systems. The economic benefits purveyed by every reactor were modelled throughout the operational period in terms of wastewater reuse and valorization via bioelectricity generation.

5.1 Introduction

Cost-benefit analysis (CBA) is a systematic process used for the calculation and comparison of the costs and benefits of any project or policy. Previously researchers have applied the CBA model in Serbia to assess the investments of a wastewater treatment plant (Djukic et al., 2016), and in Spain for analyzing the biofuel consumption targets (Santamaría and Azqueta, 2015). One major aspect of an effective decentralized wastewater treatment scheme is the economic sustainability during the operational period. Economically sustainable technology should produce the highest cost-benefit during operation while consuming minimal resources. Very few articles are available in the literature that compare the long-term economic aspects of standalone CW and MFC reactors with integrated planted and unplanted CW-MFC systems.

The focus of this study was to apply and explore the applicability of the CBA model to reactor R1 (vertical CW), reactor R2 (integrated CW-MFC), and reactor R3 (unplanted CW-MFC); for long-term treatment of low-strength domestic wastewater. The model was applied for Phase 1 (HRT 36 h) and Phase 2 (HRT 24 h) of the study since these two HRTs were rendered critical for the current research as explained in Section 4.4 of Chapter 4. Cost-benefit ratio (CBR) was analyzed to determine the most economically viable technology. The CBA model was strengthened further using a sensitivity analysis for the fundamental model parameters and by introducing a correlation study to understand the mutual relationship among the model parameters.

5.2 Materials and methods

Xu et al. (2018), developed a simplified CBA model for different types of MFCs, which has been implemented in the current study. The cost model and the benefit model were developed separately for all the reactors to calculate the cost-benefit ratio.

5.2.1 Experimental setup

The current study incorporates similar reactor arrangements, configurations, wetland macrophytes, and raw wastewater as were previously outlined in Chapter 2, Section 2.2.2. Comparative CBA analysis was done for 300 days in the duration of Phase 1 and Phase 2 of the study and all the annual cost-benefit analyses were performed assuming 300 days of reactor operation per annum.

5.2.2 Cost Analysis

This model comprises net investment cost, cost for operation and maintenance, and cost of unit power generation. The detailed cost analysis is given in Appendix D, Tables D1 – D3.

Net investment cost (C_{ni})

The net investment cost is expressed as the difference between the total investment cost and the depreciated material value of the reactor's post-service life.

Operation and maintenance cost (C_{mo})

This includes the labour cost for reactor assembling and material processing, the cost of water quality monitoring, the cost of regular reactor maintenance, and the cost of electricity consumption for pump operation. The cost of water quality monitoring includes reagent cost, cost of instruments handling and operation, and laboratory consultation fee. Regular reactor maintenance will include the cost of replaced materials (if any). The cost of electricity for pump operation was determined assuming 6 days of pump operation per week.

Cost of unit power generation (C_{pu})

This is the cost of a unit electrode area (1 m^2) generating unit power (1 W) while treating wastewater for 1 day. This cost was calculated using the equation given below (Xu et al., 2018),

$$C_{pu} = \frac{C_{ni}}{P \times 300} + \frac{C_{mo}}{300} \tag{5.1}$$

Where, C_{ni} and C_{mo} are the net investment cost and the operation and maintenance cost in Indian Rupee (₹), for a complete Phase of 300 days. P is the power density of the reactors (R2 and R3) in W/m^2, and the unit of C_{pu} is expressed as ₹/[(W/m^2)/d]. For reactor R1, theoretically, C_{pu} is infinite as there is no power generation.

5.2.3 Benefit analysis

The major sources of benefits that are incorporated in the current study are (i) Revenue from wastewater treatment, (ii) Revenue from bioelectricity production, and (iii) Revenue per unit of power production. The detailed cost analysis is given in Appendix D, Tables D4 – D5.

Benefit from wastewater treatment (R_{tw})

Revenue from the treatment of wastewater was estimated for a phase of 300 days by the following equation (Xu et al., 2018).

$$R_{tw} = V \times \frac{300}{T} \times \textit{Revenue from treating } 1\ m^3 \textit{ wastewater} \times 10^{-6} \qquad (5.2)$$

Where V is the effective reactor volume in L and T is the HRT in days.

Benefit from bioelectricity production (R_{pe})

The cost-benefit from bioelectricity was calculated for reactor R2 and reactor R3, using the power density and the electrode (anode) area. Revenue from bioelectricity generation has been calculated by the following equation (Xu et al., 2018),

$$R_{pe} = P \times A \times 300 \times 24 \times \textit{Price of unit electricity} \qquad (5.3)$$

Where A is the electrode area in m^2 and R_{pe} is the revenue from total power produced in a phase of 300 days in Indian Rupee (₹).

Benefit per unit power produced (R_{pu})

Benefit per unit power production was evaluated for 1m^2 electrode area that produces 1 W electricity while treating wastewater for 1 day; using the following equation (Xu et al., 2018),

$$R_{pu} = \frac{R_{pe} + R_{tw}}{P} \qquad (5.4)$$

Where R_{pu} is expressed as ₹/[(W/m^2)/d].

5.2.4 Cost-benefit ratio (CBR)

CBR is expressed as a ratio of project cost and benefits obtained in monetary terms. The CBR is often considered a concluding factor for assessing the economic sustainability of a project (Coquillard et al., 2012). A better investment of cost-benefit will exert a lower CBR value. For the current study, CBR was calculated using the following equation (Xu et al., 2018),

$$CBR = \frac{C_{ni}}{R_{pe} + R_{tw}} \tag{5.5}$$

5.2.5 Sensitivity analysis

One-at-a-time sensitivity analysis for CBR in the current study was performed using the following formula prescribed by Lanyon et al. (2021).

$$S_x = \frac{dC/C}{dX/X} \tag{5.6}$$

Where S_x is the sensitivity score of individual input parameters. C stands for the obtained CBR value after model simulation, X is the parameter that acts as the variable, dC is the change in the model output value and dX represents the change in the input parameter. Sensitivity for CBR was calculated considering net investment cost (C_{ni}), power density (P), electrode area (A), effective reactor volume (V), and HRT (T) as the input parameters. S_x for CBR was obtained by varying each parameter for 100 points which are equally distributed throughout the range of the parameter space obtained in the current study.

5.3 Results and Discussion

5.3.1 Cost model analysis of the reactors without effluent disinfection

Figure 5.1 shows the cost model analysis of reactors R1, R2, and R3 for Phase 1 and Phase 2 of the study without effluent disinfection. The net investment cost (C_{ni}) was obtained assuming a 90% depreciation of the materials value after the service life of the reactors (Xu et al., 2018). C_{ni} for reactor R1 was lower than reactors R2 and R3. This was due to the absence of electrode materials, circuit arrangement, and resistors in the reactor R1. C_{ni} for reactor R1 was obtained as ₹1409.09 and for R2 and R3 was obtained ₹1699.11. Reactor R2 and reactor R3 exerted identical C_{ni} as they were similar in configurations and there was no cost associated with the collection and use of wetland macrophyte *Typha angustifolia* as those were locally available. For 24 h HRT (Phase 2), the C_{ni} for reactors R1, R2, and R3 were rupees 440.92, 730.94, and

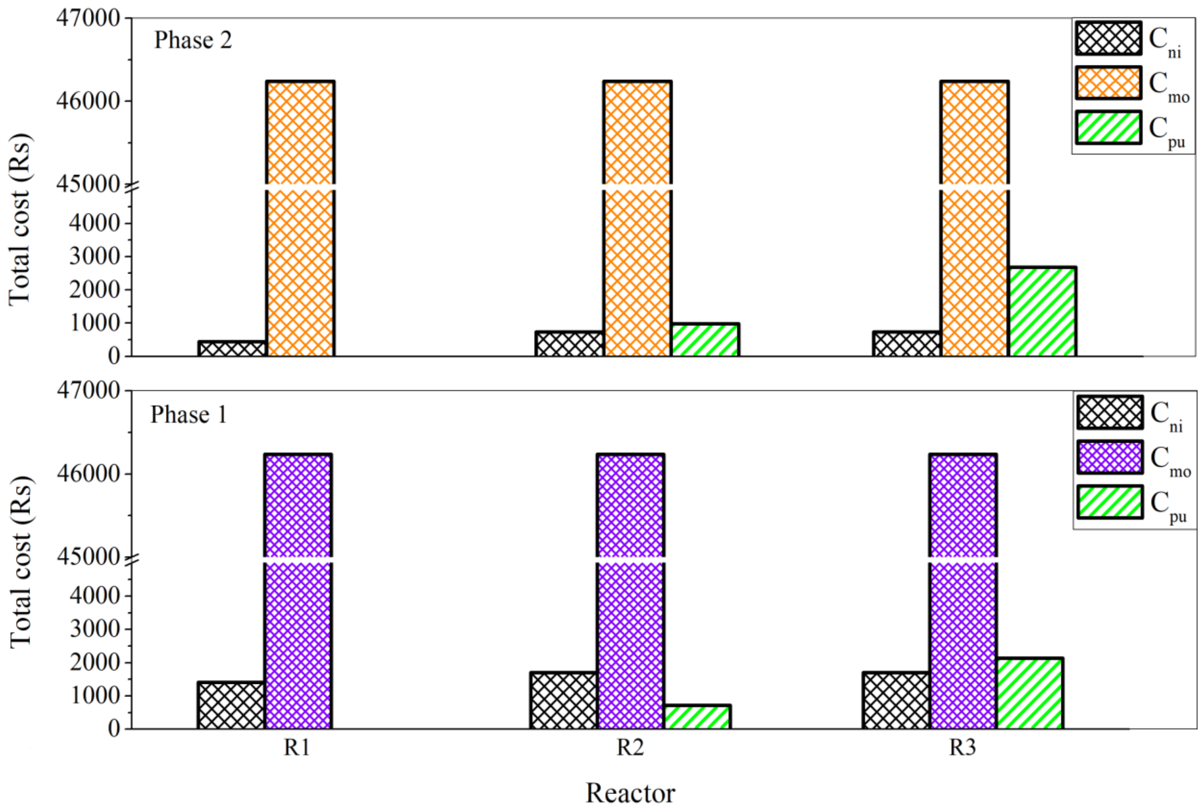

Fig. 5.1 Cost model analysis for Phase 1 and Phase 2 without effluent disinfection

730.94 respectively. The reduced C_{ni} was obtained only due to the lower area requirement for reactor operation with 24 h HRT. The reduction in the area requirement in Phase 2 was found by deciding the reduced effective volume of each reactor keeping the flow rate and effective reactor height similar to that of Phase 1 (36 h HRT). The reduced area was obtained by dividing the revised effective volume of the reactors by the reactor height. Table 5.1 summarizes the quantification of net investment cost including the cost of the materials used for reactor fabrication in the current study. Table 5.2 encapsulates the comparative assessment of the construction cost of the reactors used in the current study with that of typical MFC reactor configurations available in the literature (Jadhav et al., 2021).

The operation and maintenance cost (C_{mo}) is one fundamental part of the cost model. In the current study, C_{mo} for reactors R1, R2, and R3 were found to be rupees 46239.20 for 300 days of reactor operation (per annum). Annual C_{mo} was identical for both the phases of 36 h and 24 h HRT. The labour cost for reactor assembling and maintenance was calculated for two days per annum, one day for reactor assembling and one day for maintenance. Maintenance frequency was kept once a year since no problems related to the reactor clogging were observed within 300 days of annual operation in every phase. The standard daily labour cost for unskilled

Table 5.1 Analysis of the net investment cost of the reactors for Phase 1 & Phase 2

Reactor	Items	Unit price (₹)	No. of units	Cost (₹)	Depreciation
R1 (Phase 1)	Gravel	2.5/L	4.5 L	11.25	90 %
	PVC pipe	450/m	0.4 m	180	
	SSM	467/m^2	Nil	0	
	Graphite rod	150	Nil	0	
	GAC	319/kg	0.735 kg	234.5	
	Copper wire	8.9/m	Nil	0	
	Cost of land	3242/ft^2	0.34 ft^2	1102.28	
	Acrylic sheets	602/m^2	0.0625 m^2	37.63	
			Total investment cost	**1565.66**	
			Net investment cost	**1409.09**	
R2 (Phase 1)	**Item**	**Unit price (₹)**	**No. of units**	**Cost (₹)**	**Depreciation**
	Gravel	2.5/L	4.5 L	11.25	90 %
	PVC pipe	450/m	0.4 m	180	
	SSM	467/m^2	0.04 m^2	18.68	
	Graphite rod	150	2	300	
	GAC	319/kg	0.735 kg	234.5	
	Copper wire	8.9/m	0.4 m	3.56	
	Cost of land	3242/ft^2	0.34 ft^2	1102.28	
	Acrylic sheets	602/m^2	0.0625 m^2	37.63	
			Total investment cost	**1887.90**	
			Net investment cost	**1699.11**	
R3 (Phase 1)	**Item**	**Unit price (₹)**	**No. of units**	**Cost (₹)**	**Depreciation**
	Gravel	2.5/L	4.5 L	11.25	90 %
	PVC pipe	450/m	0.4 m	180	
	SSM	467/m^2	0.04 m^2	18.68	
	Graphite rod	150	2	300	
	GAC	319/kg	0.735 kg	234.5	
	Copper wire	8.9/m	0.4 m	3.56	
	Cost of land	3242/ft^2	0.34 ft^2	1102.28	
	Acrylic sheets	602/m^2	0.0625 m^2	37.63	
			Total investment cost	**1887.90**	
			Net investment cost	**1699.11**	

Table 5.1 continued…

Reactor	Items	Unit price (₹)	No. of units	Cost (₹)	Depreciation
R1 (Phase 2)	Gravel	2.5/L	3 L	7.5	90 %
	PVC pipe	450/m	0.4 m	180	
	SSM	467/m^2	Nil	0	
	Graphite rod	150	Nil	0	
	GAC	319/kg	0.17 kg	5.42	
	Copper wire	8.9/m	Nil	0	
	Cost of land	3242/ft^2	0.08 ft^2	259.36	
	Acrylic sheets	602/m^2	0.0625 m^2	37.63	
			Total investment cost	**489.91**	
			Net investment cost	**440.91**	
	Item	**Unit price (₹)**	**No. of units**	**Cost (₹)**	**Depreciation**
R2 (Phase 2)	Gravel	2.5/L	3 L	7.5	90 %
	PVC pipe	450/m	0.4 m	180	
	SSM	467/m^2	0.04 m^2	18.68	
	Graphite rod	150	2	300	
	GAC	319/kg	0.17 kg	5.42	
	Copper wire	8.9/m	0.4 m	3.56	
	Cost of land	3242/ft^2	0.08 ft^2	259.36	
	Acrylic sheets	602/m^2	0.0625 m^2	37.63	
			Total investment cost	**812.15**	
			Net investment cost	**730.94**	
	Item	**Unit price (₹)**	**No. of units**	**Cost (₹)**	**Depreciation**
R3 (Phase 2)	Gravel	2.5/L	4.5 L	7.5	90 %
	PVC pipe	450/m	0.4 m	180	
	SSM	467/m^2	0.4 m2	18.68	
	Graphite rod	150	2	300	
	GAC	319/kg	0.735 kg	5.42	
	Copper wire	8.9/m	0.4 m	3.56	
	Cost of land	3242/ft^2	0.34 ft^2	259.36	
	Acrylic sheets	602/m^2	0.0625 m^2	37.63	
			Total investment cost	**812.15**	
			Net investment cost	**730.94**	

labours working in water supply and sewerage lines was adopted from the order of minimum wages published by the Ministry of Labour and Employment, Government of India (see Fig. D1 in Appendix D). The cost of effluent quality checking was calculated according to the standard rates of the Central Pollution Control Board, India (CPCB, 2022).

Effluent quality checking was estimated to be carried out at an interval of 50 days for both phases keeping in mind that there might be unavailability of laboratory facilities in most rural regions and this task of analysis would have to be outsourced. The cost of electricity for operating the peristaltic pumps was measured for 26 days of pump service per month. Peristaltic pumps (Unigenetics, BT-50s) were used in the current study with a power rating of 24 W. The cost of electricity was calculated as per the electricity tariff of rupees 145/kWh (AERC, 2023). The cost of unit power generation (C_{pu}) for reactor R2 was much lower compared to reactor R3 for both 36 h and 24 h HRT. The major reason behind this is the higher power density obtained from planted CW-MFC (R2) compared to unplanted CW-MFC (R3). During 36 h HRT, the maximum power density obtained from R2 and R3 was 4.65 and 1.32 mW/m^2. With HRT of 24 h, the maximum power density for R2 and R3 was reduced to 2.96 and 0.44 mW/m^2.

Table 5.2 Comparison of the construction cost of R1, R2, and R3 with typical MFCs

Reactor	Effective volume	Anode material	Construction cost (in 1000)	Reference
R1 (CW)	4.5 L	SSM + GAC	1.57 ₹	Current study
R2 (CW-MFC)	4.5 L	SSM + GAC	1.89 ₹	Current study
R3 (unplanted CW-MFC)	4.5 L	SSM + GAC	1.89 ₹	Current study
Single MFC	20 L	Carbon fiber cloth	59.47 ₹	(Lu et al., 2017)
Tubular MFC module	200 L	Carbon brush	500.86 ₹	(Ge and He, 2016)
Stacked MFCs	600 L	SSM+Granular carbon	272.57 ₹	(Valladares et al., 2019)
Modular MFCs	1000 L	Coal GAC	2973.46 ₹	(Liang et al., 2018)

₹: Indian rupees, the construction cost of typical MFCs was converted from USD to Indian rupees using a conversion factor of 1 USD = 82.6 ₹

5.3.2 Analysis of the benefit model without effluent disinfection

Figure 5.2 shows the benefit model obtained for Phase 1 and Phase 2 of the study without effluent disinfection. Two major sources of benefit in the current chapter were the revenue from electricity production (R_{pe}) and the revenue from the treatment of wastewater (R_{tw}). R_{pe} was obtained from reactors R2 and R3 which were capable of producing bioelectricity. R_{pe} was more in R2 compared to R3 in both Phase 1 and Phase 2, due to the higher power density obtained in reactor R2 (see Chapter 4, Table 4.3). R_{tw} depends on the successful reuse of treated wastewater for either agricultural, industrial, or recreational purposes. For such conditions, the effluent quality in the current study was compared to the irrigation water quality standards in India (BIS, 2001). In the year 2004, the Ministry of Urban Development India endorsed the permissible FC limit in treated wastewater to be 2500/100 mL, for safe disposal and unrestricted irrigation reuse (Seth, 2016). The quality of treated wastewater during Phase 1 and Phase 2, have been summarized in Table D7 in Appendix D. To convert the benefit of treated wastewater into monetary terms, it was compared with the irrigation requirement of rice which has the largest area under irrigation in India. For rice, the cost of irrigation water in India was adopted as ₹ 5.19/m^3 (Upadhyaya and Roy, 2020). In the case of reactor R1, the effluent quality of R1 did not comply with the required irrigation water quality in terms of FC concentration without effluent disinfection (for both Phase 1 and Phase 2) and no benefit was obtained from the bioelectricity production.

During the first phase of the study with 36 h HRT, only reactor R2 was capable of producing the effluent which was satisfying the fecal coliform (FC) limit with other physicochemical parameters of irrigation water. Effluent from reactors R1 and R3 failed to conform to the fecal coliform standards though other parameters were satisfactory for irrigation reuse. In Phase 2 with 24 h HRT, no reactor effluent was found suitable for irrigation as the fecal coliform was higher in all three. Figure 5.2 shows that no benefit from reactor R1 was obtained in both phases of the study without effluent disinfection. Reactors R2 and R3 were only capable of providing benefits of bioelectricity production during Phase 2 of the study. During Phase 1, the benefit from bioelectricity production (R_{pe}) from reactors R2 and R3 were rupees 0.38 and 0.11 per annum. R_{tw} from reactor R2 was rupees 4.67 per annum. Revenue per unit power production (R_{pu}) from reactor R1 in Phase 1 was zero as there was no bioelectricity generated and the effluent was not compatible with irrigation standards. R_{pu} from reactor R2 was 1086.24

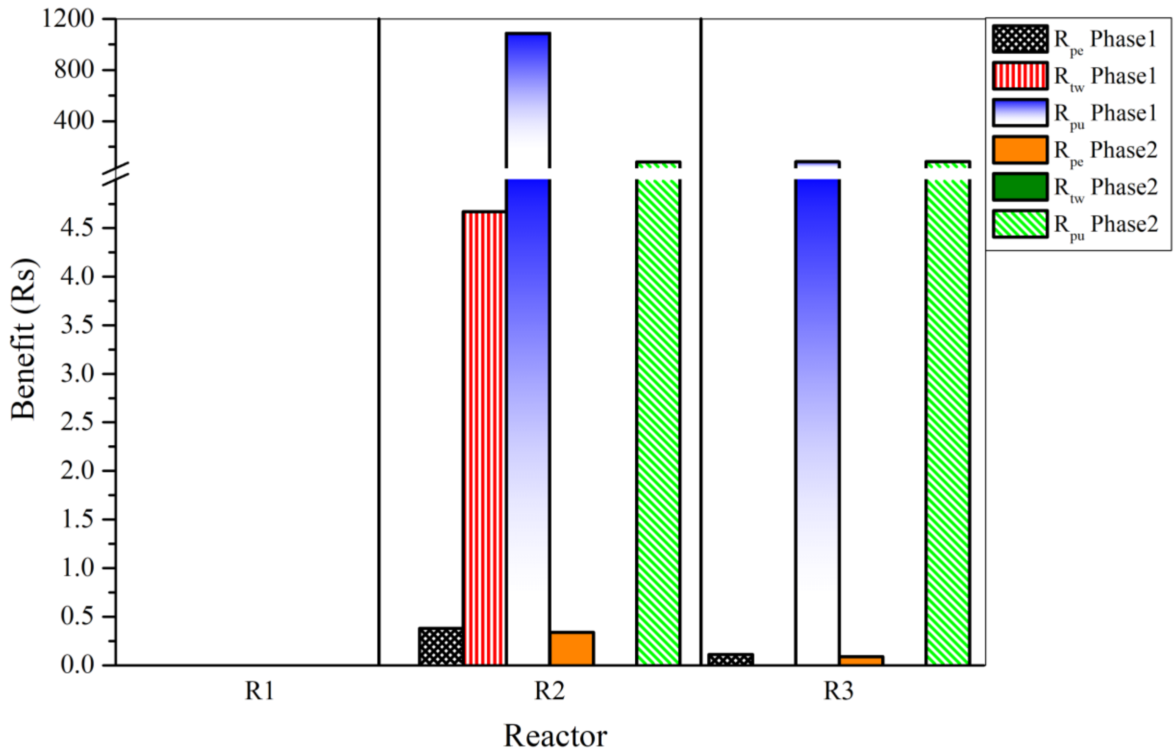

Fig. 5.2 Benefit model analysis of Phase 1 and Phase 2 without effluent disinfection

₹/(W/m^2) per annum, which was comparatively higher than reactor R3 with R_{pu} of 81.82 ₹/(W/m^2) per annum. In Phase 2, the R_{pu} value of R1, R2, and R3 was found to be zero, 81.93, and 84.11 ₹/(W/m^2) per annum, respectively. The reduction in revenue was due to low-quality effluent produced by all the reactors. With lower HRT, bioelectricity production was hampered that resulted in lower power density from reactor R2 and reactor R3 in Phase 2.

5.3.3 Modification of the cost-benefit model by introducing effluent disinfection

In the current research, one major part of the monetary benefit was obtained from the reuse of treated wastewater for irrigation purposes. Fecal coliform was a critical parameter that restricted the reuse of the effluents from reactor R1 and reactor R3 in Phase 1. During Phase 2, the presence of fecal coliforms in the treated effluents from R1 and R3 reactors was detected to be beyond the desirable limit. This restricted the advantage of benefits from wastewater treatment although the net invested cost was significantly reduced in Phase 2 (as seen in Table 5.1). So, a cost-benefit analysis was done further for Phase 1 and Phase 2 of the study by introducing disinfection of the effluents of all the reactors. Disinfection resulted in an increase in the operation and maintenance costs of the reactors. The cost of effluent disinfection was assumed to be ₹ 0.076/L of treated water for chlorination followed by dechlorination in the Indian scenario (Tak and Kumar, 2017).

The modified cost model for Phase 1 and Phase 2 has been illustrated in Figure 5.3 (a). With effluent disinfection in place, the operation and maintenance cost (C_{mo}) for reactors R1, R2, and R3 increased to Rs. 46307.6 per annum in Phase 1 and Phase 2; from Rs. 46239.2 per annum without disinfection. The net investment cost (C_{ni}) and the cost of unit power generation (C_{pu}) were not affected in both phases due to the introduction of the effluent disinfection process. The introduction of effluent disinfection improved the overall benefit model in Phase 1 and Phase 2. Figure 5.3 (b) shows the modified benefit model of Phase 1 and Phase 2, for all the reactors. With disinfection, revenue from wastewater treatment could be availed in all the reactors since a combined chlorination and dechlorination process is expected to raise the final effluent quality of the reactors after the initial reactor treatment. The final effluent should be fit for irrigation reuse. The amount of revenue from wastewater treatment (R_{tw}) in all the reactors was found to be ₹ 4.67 per annum, which was identical for both Phase 1 and Phase 2.

5.3.4 Cost and benefits associated with unit power production

During Phase 1, without disinfecting the reactor effluents, the cost of unit power generation (C_{pu}) for reactor R3 (unplanted), was found to be 2138.02 ₹/[(W/m^2)/d], which was much greater than planted reactor R2 which reflected C_{pu} value of 717.30 ₹/[(W/m^2)/d]. In Phase 2, the observed C_{pu} for reactor R2 and reactor R3 was 981.06 and 2677.53 ₹/[(W/m^2)/d]. The presence of plants in the reactor helped to increase the power density (as mentioned in previous chapters) which is the major reason that reactor R2 has exerted a low C_{pu} value compared to that of reactor R3. With the reduction of HRT from 36 h to 24 h in Phase 2, both reactors showed an increase in C_{pu} value due to a depletion in power density. The addition of effluent disinfection increased the operation and maintenance cost (C_{mo}), which influenced the cost of unit power production as well.

However, in the current study, the application of effluent disinfection seemed to have minimal effects on the C_{pu} value for reactors R2 and R3 (Table D3 in Appendix D) and power density emerged as the critical factor governing C_{pu}. During Phase 1, without disinfection of the reactor effluents, the revenue from wastewater treatment (R_{tw}) was zero for reactor R3. This led reactor R3 to exert very low revenue per unit power production (R_{pu}), compared to reactor R2, despite R2 yielding much higher power density. In Phase 2, with reduced HRT, the R_{pu} value for R3 was lower than R2. The scenario changed when effluent disinfection was applied to attain revenue from wastewater treatment. The R_{pu} value for reactor R3 increased nearly 3.3

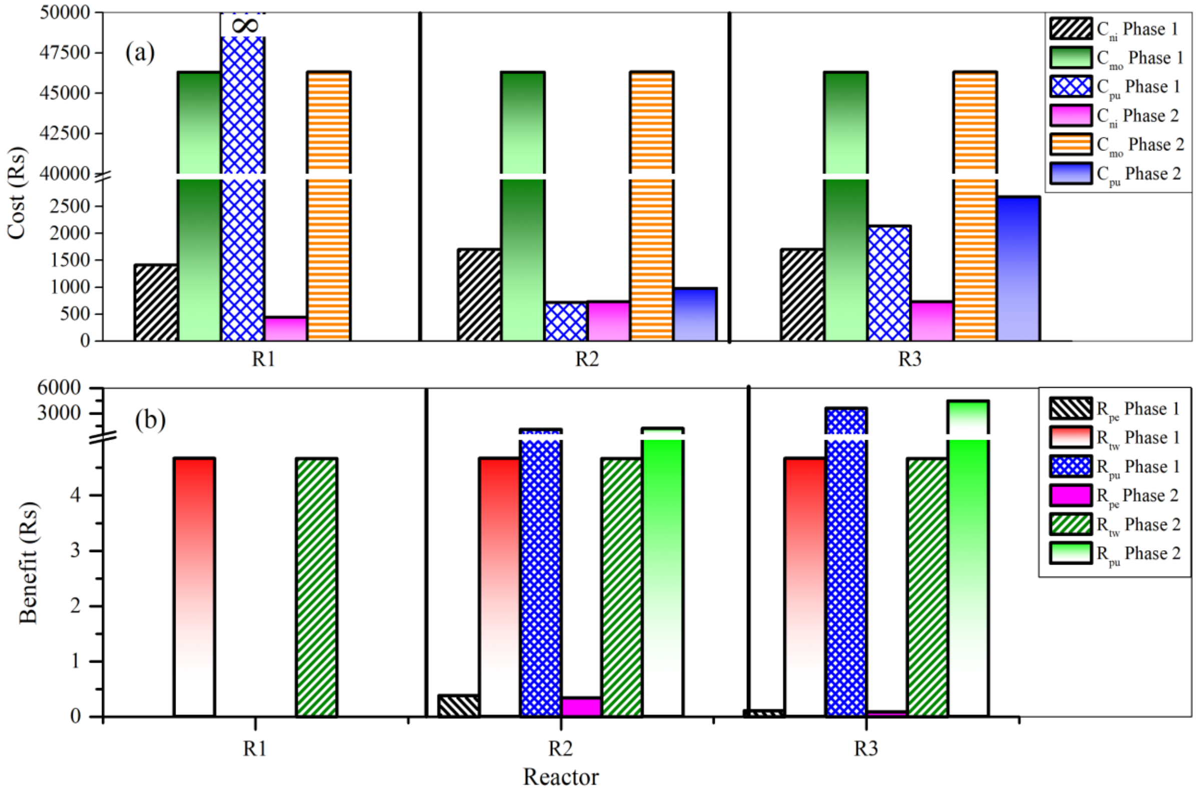

Fig. 5.3 Cost model for Phase 1 and Phase 2 with effluent disinfection, (b) benefit model for Phase 1 and Phase 2 with effluent disinfection

times in Phase 1 and 3.7 times in Phase 2, compared to that of reactor R2 when revenue from wastewater treatment was attainable (see Table D4 and D5 in Appendix D). These results indicate that R_{tw} is a significant parameter that can influence the revenue from power production and the effect of lower power density can be surpassed by the effluent disinfection strategy.

5.3.5 Cost-benefit ratio

Table 5.3 shows the cost-benefit ratio (CBR) obtained for reactors R1, R2, and R3 in Phase 1 and Phase 2; calculated as per Equation 5.5. Effluent disinfection and HRT seemed to be two critical factors affecting the CBR in the current study. Effluent disinfection affected the revenue generated from wastewater treatment (R_{tw}), and variation in the HRT affected the net investment cost (C_{ni}) of the reactors used. Without effluent disinfection, reactor R1 exerted a CBR value of infinity during Phase 1 and Phase 2, which indicated that the application of R1 would not be giving any economic benefit in monetary terms. CBR became infinity as reactor R1 was incapable of producing bioelectricity which rendered revenue from electricity production (R_{pe}) value as zero, and without disinfection, the effluent reuse for irrigation was not feasible which rendered R_{tw} value to zero. This turned the denominator ($R_{pe}+R_{tw}$) in CBR = 0, and the result obtained was infinity. Without effluent disinfection, the CBR value was lower for reactor R3 in Phase 2 compared to Phase 1 due to a reduction in the net investment cost (C_{ni}) because reduced HRT from 36 h to 24 h led to less areal footprint of the reactors. When effluent disinfection was not in place, the application of reactor R2 was found to be the most economical solution for wastewater treatment as it rendered minimum CBR values for Phase 1 and Phase 2 among all three different reactors.

The CBR scenario changed when effluent disinfection was applied to all the reactors. Reactor R2 rendered identical CBR values with and without effluent disinfection. The reason for this was the capability of reactor R2 to reduce the fecal coliform load in the effluent even without disinfection with adequate contact time available in Phase 1. Introducing the disinfection process in the cost-benefit model did not alter the revenue value from wastewater treatment (R_{tw}) in the case of reactor R2. It can be observed from Table 5.3, that reactor R3 produced a higher CBR value compared to reactor R2 in both phases, irrespective of the application or non-application of effluent disinfection. It implies that the application of standalone MFC or unplanted CW-MFC can never surpass the economic benefits offered by integrated or planted CW-MFC reactors for the treatment of domestic wastewater. This result also establishes that the presence of macrophytes can be economically beneficial as the plants were the only difference between the design configuration of reactor R2 and reactor R3.

The rhizome network of wetland macrophytes (*Typha angsutifolia*) is responsible for root-level oxygen release which results in better treatment of the wastewater by increasing the redox gradient of the reactor. An interesting observation was obtained in the case of reactor R1 after the inclusion of the effluent disinfection process in the model. The CBR value of reactor R1 dropped to the minimum among all three reactors in both phases, suggesting that installation and application of standalone constructed wetlands can be the most beneficial domestic wastewater treatment strategy when associated with the disinfection process for the production of high-quality coliform-free effluent. The primary reason that reactor R1 showed lower CBR compared to R2 was the low C_{ni} value. The C_{ni} for reactor R2 was 17% and 37.61% higher than reactor R1 in Phase 1 and Phase 2 respectively. The major reason behind this was the cost of installing electrodes and circuit arrangements for the integrated R2 system. Since the benefit from wastewater treatment was identical for both R1 and R2 after disinfection, it was necessary for reactor R2; to generate a sufficient amount of bioelectricity to increase the R_{pe} value to such an extent so that it could neutralize the additional cost of electrode arrangements.

In general, it is difficult to correlate the CBR results obtained from lab-scale models with those of full-scale reactors installed in field conditions (Jadhav et al., 2021). In the available works of literature, most studies have been performed with synthetic wastewater, which includes the cost of reagents used for preparing the wastewater. In the current study, raw domestic sewage was used as the reactor feed for adhering to the field experimental conditions and omitting the hidden costs associated with lab-scale studies. However, it is important to understand that CBR will always depend on several other factors like wastewater components, biochemical and chemical oxygen demand, nutrient concentration, reactor size, processing cost, reactor power density, etc. Reactors that are fed with wastewater having either acetate or glucose as the carbon source will exert high power density compared to the reactors that are fed with raw complex wastewater containing bio-recalcitrant organics. Table 5.4 gives a comparative analysis of CBR values reported by Xu et al. (2018), for different types of CW-MFC reactors while treating synthetic wastewater. However, it is worth mentioning that the referred CBR values given are mainly based on literature surveys and theoretical assumptions. In the current study, a lower CBR value was obtained with comparatively less power density and HRT. The use of synthetic wastewater is a probable reason for the higher power density obtained by previous studies. Oon et al. (2016) and Xu et al. (2018), obtained CBR values close to the current study with similar electrode materials, which is indicative of the dependence of CBR on the class of electrodes. It is easily understood that different types of electrodes will produce different power densities (Gupta et al., 2021). Since CBR can be influenced by so many factors associated with the reactor characteristics, influent, and effluent water quality which in turn can affect the costs and benefits associated with them; sensitivity analysis for CBR including fundamental parameters was deemed necessary for the current study.

Table 5.3 CBR values for Phase 1 and Phase 2 with and without effluent disinfection

CBR	Without effluent disinfection			With effluent disinfection		
	R1	R2	R3	R1	R2	R3
Phase 1	Infinity	336	15725	301	336	355
Phase 2	Infinity	145	8122	94	145	153

5.3.6 Sensitivity study and correlation analysis of the model parameters

Sensitivity analysis was performed with 5 parameters: net investment cost (C_{ni}), power density (P), electrode area (A), hydraulic retention time (T), and effective reactor volume (V), to provide a precise idea of how the CBR is influenced by the individual model input parameter. The sensitivity score for each abovementioned parameters was calculated as per equation 5.6. Figure 5.4 (a) shows the sensitivity score obtained for each parameter in the current study as a boxplot illustration. The current CBR model was found to be most sensitive to power density (P) which indicates the importance of harvesting high power output from an integrated CW-MFC system to obtain more economical benefits. The CBR model was sensitive to the electrode area (A), which shows that the available expanse of the electrode surface can significantly affect the project outcome in terms of benefit.

Table 5.4 Comparative CBR values with literature for planted CW-MFC

Wastewater	Anode	Cathode	Power density (mW/m^2)	Power (mW)/(m^3 of wastewater)	HRT (d)	CBR	Reference
Raw domestic sewage	SSM with graphite rod and GAC	Graphite rod with activated carbon	4.65	140.33	1.5	145	Current study
Raw domestic sewage	SSM with graphite rod and GAC	Graphite rod with activated carbon	4.20	77.33	1	336	Current study
Synthetic wastewater	Titanium mesh with activated carbon	Titanium mesh with activated carbon	3714	58310	3	332	Xu et al., 2018
Synthetic wastewater with glucose as carbon source	Activated carbon	Stainless steel with activated carbon	74.45	9130	3	249	Fang et al., 2017
Synthetic wastewater with acetate as carbon source	SSM with activated carbon	SSM with activated carbon	9.30	4649	1	253	Oon et al., 2016
Synthetic wastewater with glucose as carbon source	Carbon felt	Platinum loaded carbon paper	27.50	12	8	82923	Wu et al., 2013

SSM: stainless steel mesh, GAC: granular activated carbon, HRT: hydraulic retention time

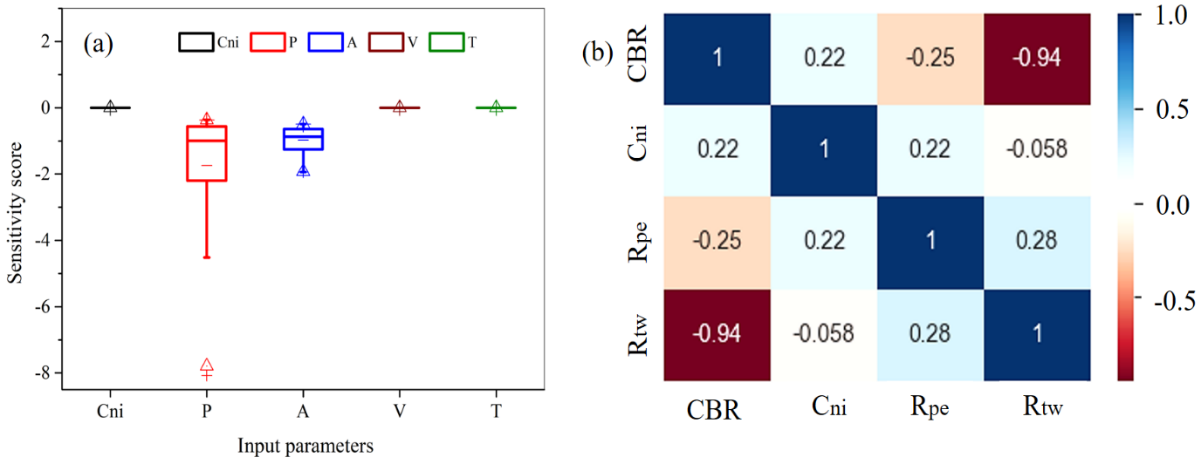

Fig. 5.4 (a) Sensitivity analysis of individual model parameters with CBR output, (b) Correlation matrix of CBR, C_{ni}, R_{pe}, and R_{tw}

Trapero et al. (2017), performed a sensitivity study on the MFC reactor to check the industrial application potential and found electrode area to be one major influential parameter. However, the sensitivity analysis was limited to the electrode area and the annual growth rate of electricity price only. In the current study, correlation analysis was also implemented for the current CBR model with C_{ni}, R_{pe}, and R_{tw}, as the input parameters. Figure 5.4 (b) shows the correlation matrix of these four parameters. It is visible that C_{ni} has a positive correlation with CBR, while R_{tw} and R_{pe} showed a negative correlation, which is evident from Equation 5.5. R_{tw} imparted a high positive correlation with the CBR which means an elevation in R_{tw} value can lead to a significant reduction in CBR value. C_{ni} and R_{pe} showed a positive correlation between them indicating a potential rise in the investment cost for obtaining higher bioelectricity production. R_{tw} and R_{pe} also showed a positive correlation among them which suggests that higher bioelectricity generation can be an indication of better effluent quality while other parameters are kept constant.

5.4 Conclusion

During Phase 1 and Phase 2, planted CW-MFC (R2) appeared as the most sustainable option with minimum CBR value for domestic wastewater treatment. This was possible as revenue from electricity production (R_{pe}) and revenue from wastewater treatment (R_{tw}) could be achieved due to its inherent coliform removal capability. The major role of effluent disinfection was to achieve the benefits from treated wastewater reuse in irrigation after removing the fecal coliforms. Disinfection was found necessary for unplanted CW-MFC (R3) and CW (R1) reactors in Phase 1 and particularly in Phase 2 for all the reactors for effluent reuse. With effluent disinfection in place, R1 (planted with no electrodes) achieved lower CBR values as compared to R3 (unplanted with electrodes) in both Phase 1 and Phase 2. This shows that the presence of macrophytes alone is economically more beneficial as compared to electrodes without plants. Based on this, plants could be recommended for future reactor design to achieve a low CBR ratio during domestic wastewater treatment.

Sensitivity analysis revealed power density to be the most sensitive input parameter. Producing higher power density in Phase 1 and Phase 2 also strengthens the argument for applying reactor R2 without effluent disinfection. After the application of effluent disinfection, reactor R1 surpassed reactors R2 and R3 in terms of economic advantages as the investment cost was much lower for R1. The correlation matrix showed, that R_{tw} has a high negative impact on the CBR. Effluent disinfection was not beneficial for reactor R2 in Phase 1 as it did not improve the model benefit, rather it increased the operation and maintenance cost. With HRT reduced to 24 h, the area required for reactor installation decreased, which benefited the revenue model and reduced the CBR. An interesting observation from the correlation matrix was that R_{pe} and C_{ni} were positively correlated which means, that if power output is to be increased, it will enhance the net investment cost. Therefore, it would be challenging for future environmental engineers to achieve substantial economic sustainability by optimizing these parameters for the operation of integrated CW-MFC systems during wastewater treatment.

In this study, the effort to make the CBA model as practical as possible was prioritized by using raw domestic wastewater instead of synthetic wastewater and carefully analyzing the local factors like labour cost, cost of land, electricity tariff, cost of effluent quality check, etc. The benefits achieved from the treated wastewater in all the reactors were considered identical after the application of the disinfection process. However, it was seen in Chapter 4 that the quality of the reactor effluents degraded with reduced hydraulic retention time. Thus the positive correlation between R_{pe} and R_{tw} becomes a significant outcome of this chapter. It implies that a reactor with higher power output will exert higher revenue from wastewater treatment by producing better effluent quality. It could be concluded that in the future, more beneficial and careful reuse of treated wastewater should be prioritized to maximize the economic benefits of decentralized wastewater treatment. In the case of integrated bioelectrochemical systems such as CW-MFC (reactor R2) used in this study, the key to generate economic benefit is to focus on the power density increment, which will enhance the effluent quality.

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Chapter 6

Investigation of Bioelectricity Enhancement and E. coli Inactivation from Domestic Wastewater in Multi-Anode CW-MFC Reactor

This chapter is dedicated for the assessment of the scope of using a multi-anode CW-MFC reactor for the treatment of low-strength domestic wastewater, as compared to a single-anode CW-MFC reactor. The system monitoring was done focusing on the enhancement of bioelectricity harvesting, organic removal, and inactivation of *Escherichia coli*.

6.1 Introduction

The performance of a typical CW-MFC reactor is dependent on multiple factors such as wastewater characteristics, internal resistance, electrode arrangements, the population of electroactive microbes, hydraulic retention time, etc. (Huang et al., 2021; Ebrahimi et al., 2021). The electrodes of integrated CW-MFC reactors are of particular interest as they play significant roles in governing the treatment performance and bioelectricity production. Ideally, the electrodes (the anode in particular) are expected to be compatible with the electroactive bacteria (EAB) in such a way that the EABs can attach and grow on the anode surface facilitating better anode-EAB interaction and microbial external electron transfer (Hemdan et al., 2023; Mier et al., 2021).

Fundamentally, the anode acts as an electron sink of the CW-MFC reactors during the process of bacterial substrate metabolism. A multi-anode system is capable of facilitating a larger electrode surface area for microbial attachment which ensures better interaction and direct extracellular electron transfer between EABs and anodes. Another major advantage of multi-anode reactors is that they may solve the potential challenge of scaling up the reactors for field-scale applications (Xie et al., 2024). While scaling up single anode bioelectrochemical reactors, the increase in effective reactor volume reduces the power output of the system. This happens because a single anode cannot maximize the number of electrons that could be harvested during microbial organic decomposition. The application of multi-anode reactors may also reduce the chances of voltage reversal by lowering the surface-specific internal resistance (Opoku et al., 2022).

The incorporation of multiple anodes in place of a single anode has been a major centre of attraction in the field of MFC research as it could be very influential for increased pollutant removal and bioenergy harvesting. Compared to single-anode MFC, the multi-anode MFC was successful in enhancing bioelectricity production up to 0.8 – 3.2 times (Opoku et al., 2022; Jiang et al., 2010; Xie et al., 2024). These studies have mostly incorporated carbon-based electrodes which are generally known to be less expensive, biocompatible, and equipped with considerable specific surface area. However, the application of multi-anode arrangement in integrated CW-MFC reactors is few in scientific literature. Previous CW-MFC research has primarily focused on conductivity or surface property modifications of electrodes to amplify the power output performance (Kim et al., 2019; Wang et al., 2019). Some researchers have also explored the use of composite electrodes and metal-free catalysts to enhance the treatment efficiency and bioelectricity production in CW-MFC reactors (Santoro et al., 2014; Liu et al., 2014). However, these electrode modification processes are expensive which acts as a prominent barrier towards scaling up of CW-MFC technology.

In Chapter 3 and Chapter 4, it was found that EABs played a pivotal role in organics and FC removal. The EAB-anode interaction was found essential for enhanced treatment performance of the CW-MFC reactor. The findings of Chapter 5 accentuated the economic viability of the CW-MFC system. Keeping that in mind, the current chapter was focused on the fabrication and operation of planted and unplanted multi-anode CW-MFC reactors targeting enhanced bioelectricity production while treating low-strength domestic wastewater. Another objective was the assessment of fecal coliform (FC) and *Escherichia coli* (*E. coli*) inactivation in both single and multi-anode reactors. *E. coli* is well known to be capable of extracellular electron transfer, so the comparative inactivation of *E. coli* and FC in single and multi-anode CW-MFC was of particular interest in this phase of the research along with the bioelectricity intensification.

6.2 Materials and methods

6.2.1 Reactor configuration and operation

The current chapter incorporated four lab-scale vertical up-flow reactors, each having an effective volume of approximately 4.5 – 4.6 L. The first reactor was a single-anode CW-MFC planted with *Typha angustifolia*, subsequently designated as reactor R1. The second reactor was a multi-anode CW-MFC planted with *Typha angustifolia* and subsequently designated as reactor R’1. The third reactor was an unplanted single-anode CW-MFC and the fourth reactor was an unplanted multi-anode CW-MFC subsequently designated as reactor R2 and reactor R’2 respectively. The complete reactor setup with related dimensions has been illustrated in Figure 6.1. Reactor R1 used in this study was identical to the integrated CW-MFC reactor (R2) described in the previous chapters. Reactor R2 in the current chapter was identical in configuration to the unplanted CW-MFC (R3), described in previous chapters. Reactor R’1 and reactor R’2 were equipped with three anodes namely A1, A2, and A3, and a single cathode. A1 was placed 10 cm above the reactor bottom. A2 was placed 5 cm above A1 and A3 was kept 5 cm above A2. The cathode electrode was placed 10 cm above A3. Stainless steel mesh (SSM) and graphite rods buried in granular activated carbon (GAC) were used as anode in all the reactors. Graphite rods and GAC was adopted as the cathode in reactors R1, R2, R’1, and R’2. The graphite rods were extended outside the body of the reactors to complete the external circuit connection. Medium-sized gravels (10 mm – 20 mm) were used as the drainage layer and the nonconductive filler media in between the electrodes.

The four reactors were operated in continuous up-flow mode using peristaltic pumps (BT50S). The pump flow rates were kept to maintain an HRT of 36 h. The study was conducted for 135 days. The initial 15 days were used for inoculation and acclimatization. For the next 90 days, all the reactors were operated in closed-circuit mode, and in the next 30 days, the reactors were operated in open-circuit mode. All the reactors were equipped with adequate sampling ports (designated as S1, S2, S3, and so on) for sample collection and analysis as shown in Figure 6.1.

6.2.2 Analytical methods

Samples were collected from all the reactors' effluent ports twice a week for analysis of the reactor performance. The raw wastewater characteristics were also regularly monitored with similar frequency. The wastewater and the reactor effluents were characterized by analyzing COD, redox potential (ORP), Fecal coliform (FC), and *Escherichia coli*. (*E. coli*). The COD, ORP, and FC for the raw wastewater and the reactors’ effluents were analyzed as per the methods mentioned in Section 2.2.4 of Chapter 2. *E. coli* was calculated using the multiple tube fermentation technique by applying the indole production method as prescribed in Section 9221.G in the Stanadard methods (APHA, 2017). Calculation of the hydraulic retention time

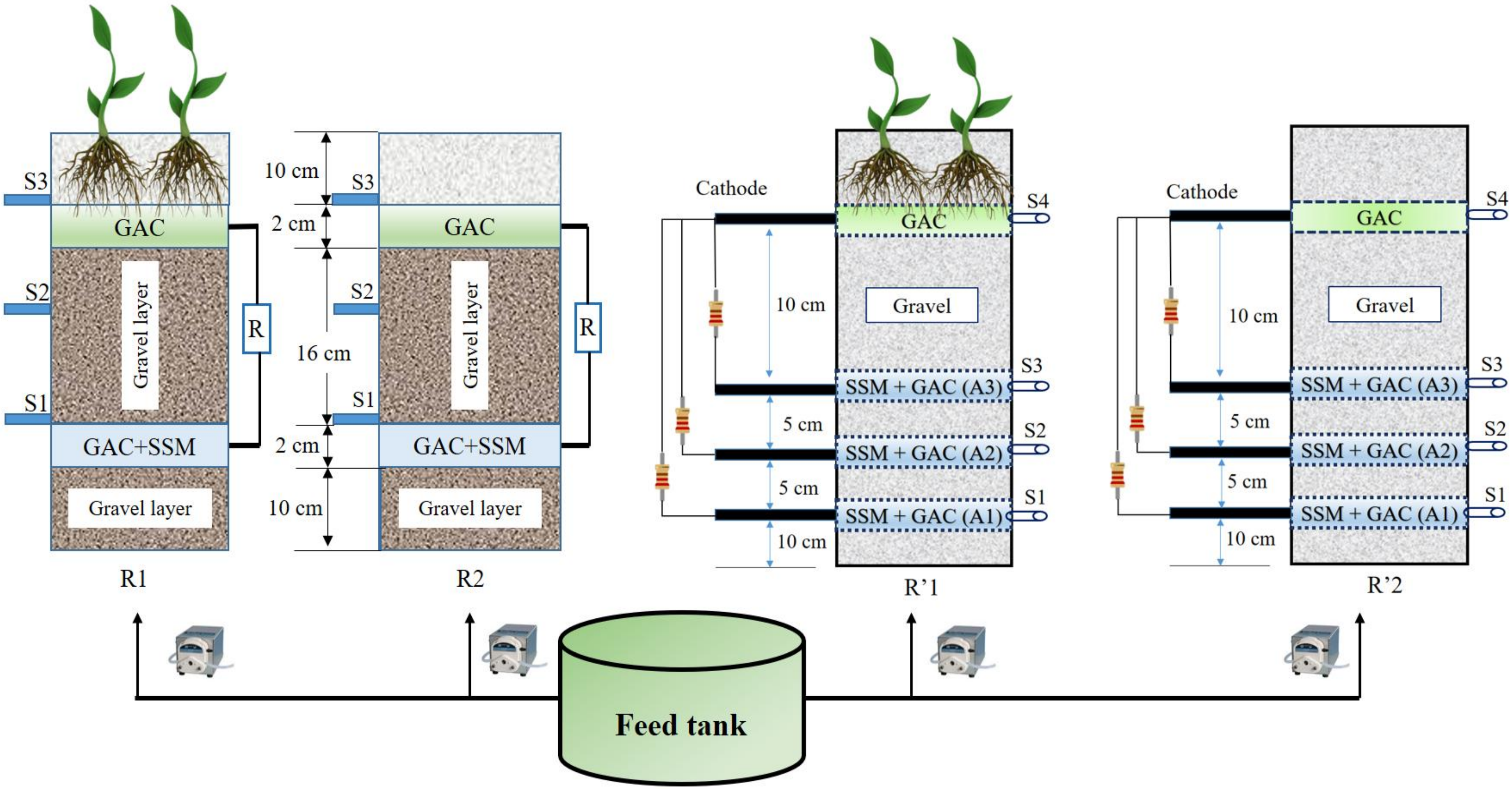

Fig. 6.1 Schematic diagram of the reactor setup

time (HRT) and COD removal efficiency, log removal of TC, FC, and *E. coli* were performed using equations 2.1, 2.4, and 2.5 respectively as described in Chapter 2. For the monitoring of the electrical performance of all the reactors, current (I), power (P), power density (PD), and current density (CD) were measured using equations 2.7, 2.8, 2.9, and 2.10 respectively as described in Chapter 2.

6.3 Results and discussion

6.3.1 COD removal from domestic wastewater

The four reactors were fed with domestic wastewater having a mean COD of 212.67±9.50 mg/L. Figure 6.2 (a) shows the variation in wastewater COD and the reactor effluents with time, during the closed-circuit and the open-circuit operations of the reactors. During closed circuit operation, single-anode planted CW-MFC (R1) produced an effluent COD of 9.32±3.35 mg/L and the unplanted single-anode CW-MFC (R2) produced an effluent COD of 25.22±4.80 mg/L. In the case of the multi-anode CW-MFCs, the planted reactor (R'1) and the unplanted reactor (R'2) exerted an effluent COD of 5.60±3.62 mg/L and 17.80±7.87 mg/L, respectively. When the reactor operation was altered to open circuit mode, the effluent COD levels were increased in all the reactors. Reactors R1, R2, R'1, and R'2 produced mean effluent CODs of 15.68±3.59 mg/L, 34.76±5.08 mg/L, 12.93±3.06 mg/L, and 33.7±8.18 mg/L, respectively. Figure 6.2 (b) shows the mean COD removal efficiencies obtained from the reactors during the closed and open circuit operation of the reactors. From Figure 6.1, fewer fluctuations could be observed in the effluent COD and the COD removal efficiencies of the reactors, irrespective of the variations in the feed COD.

This suggests that all the reactors attained a steady state after the acclimatization period. The application of GAC to embed the SSM anodes might have played an important role in producing the steady-state reactor performance due to their capabilities of handling organic and toxic shock loading (Oon et al., 2016; Sublette et al., 1982). In the current study, higher COD removal was accomplished while the reactors were operated in closed-circuit mode. This emphasizes the importance of electron transfer to the cathode via the external circuit and an unperturbed interaction of EABs with the anode for better organic degradation. Once the external circuit is opened, the complete electron transfer process is hampered and the COD degradation efficiency is reduced. Previously, Wen et al. (2021), Tang et al. (2019), and Srivastava et al. (2015) have reported similar trends during their investigation of open and closed-circuit operation of CW-MFC reactors.

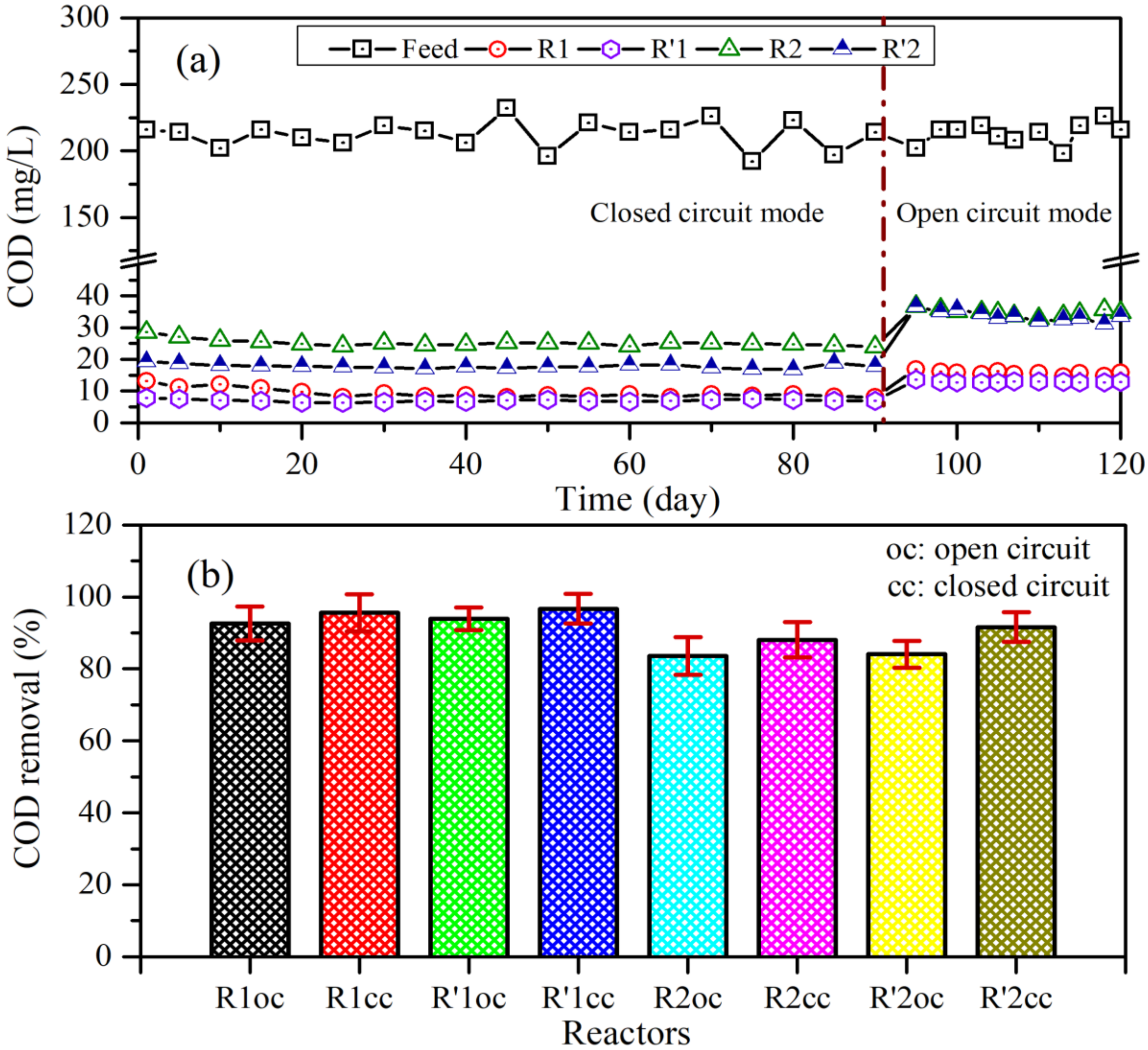

Fig. 6.2 (a) COD variation in feed and different reactor effluents during the study, and (b) Mean COD removal efficiencies exerted by different reactors.

During closed circuit operation, the mean COD removal performances of the reactors were in the order of R’1>R1>R’2>R2. A similar trend was observed during the open circuit mode but the difference in COD removal efficiency between the multi-anode and single-anode reactors was reduced. During closed-circuit and open-circuit operation, reactor R’1 exerted 1.84% and 1.40% higher COD removal efficiency as compared to reactor R1. In the case of unplanted reactors, R’2 achieved 3.9% and 0.6% higher COD removal as compared to R2 during closed-circuit and open-circuit operation. An interesting observation was that multi-anode reactors (R’1 and R’2) showed lower COD removal in open-circuit operation as compared to the closed-circuit operation of single-anode reactors (R1 and R2). This indicates the importance of the electron flow from the anode to the cathode for better wastewater treatment in CW-MFC reactors irrespective of the number of the associated anodes.

6.3.2 Spatial analysis of redox potential and dissolved oxygen

The fundamental synergistic principle of an integrated CW-MFC system is based on the natural occurrence of dissolved oxygen (DO), and redox potential (ORP) gradients between the anode and the cathode. The DO is a basic parameter that governs the aerobic and anaerobic microbial degradation pathways inside the CW-MFC reactors (González et al., 2024). The ORP on the other hand, is directly correlated with the DO concentration and is responsible for the amount of electron transfer from the anode to the cathode via the external circuit and the electrical performance of CW-MFC reactors (Srivastava et al., 2021). In the current study, the spatial DO and ORP variation was found to be mutually corresponding in all the reactors. Figures 6.3 (a) and 6.3 (b) illustrate the spatial DO variation in multi-anode and single-anode reactors respectively. Similarly, figures 6.2 (c) and 6.2 (d) show the spatial variation of the ORP in single-anode and multi-anode reactors.

The trends of the DO and the ORP profiles were approximately similar from the bottom to the top when compared between the single-anode and multi-anode reactors. In the case of the planted reactors, reactor R1(single-anode) and reactor R'1 (multi-anode) showed higher DO and ORP at the top sampling ports (S3 and S4 respectively) as compared to the bottom ports. In the effluent of the unplanted reactors R2 (single anode) and R'2 (multi-anode), the DO and ORP were found to be considerably low due to the absence of the *Typha* plants. The multi-anode reactors (both planted and unplanted) produced effluents with higher DO and ORP as compared to the single-anode reactors which was probably due to the low amount of residual organics in the effluents. For reactor R'1 (planted multi-anode CW-MFC), DO was not detected at sampling ports S1 and S2 which were kept at the level of anode A1 and anode A2 (see Fig. 6.1). The DO concentration at S3 (at the level of anode A3) and S4 (at the level of cathode) was found to be 0.23±0.06 mg/L and 2.36±0.27 mg/L. In the case of reactor R'2 (unplanted multi-anode CW-MFC), no DO was detected in sampling ports S1, S2, and S3. A very low presence of DO was observed at S4 at 0.49±0.11 mg/L. The trend was similar for the single-anode reactors R1 (planted) and R2 (unplanted) as well. For reactor R1, a sharp rise in DO concentration is visible from sampling port S2 to S3, which was also observed in reactor R'1. Reactor R2 and reactor R'2 showed little enhancement in DO content near the cathode region. At sampling port S3, the DO was 0.31±0.07 mg/L in reactor R2, while no DO was detected at S1 and S2.

Figure 6.3 (c) and Figure 6.3 (d), elucidate a constant rising trend in the ORP of the reactors from the bottom to the top. At the bottom-most sampling port (S1), ORP was observed to be below -150 mV suggesting an anaerobic environment. In reactor R'1 and reactor R1, the ORP near the cathode was found to be 83±12 mV and 79±11 mV respectively. In the unplanted reactors, the ORP near the cathode was obtained as 29±6 mV and 18±4 mV for reactor R'2 and reactor R2 respectively. At the cathode, the ORP was much higher in planted reactors as compared to the unplanted reactors. This could be attributed to the radial oxygen loss (ROL) by the roots of *Typha angustifolia* (Di et al., 2020). The *Typha* species are capable of creating a micro-aerobic environment at the rhizome network via ROL resulting in higher DO concentration and ORP at the cathode, as discussed earlier in Chapter 2, Chapter 3, and Chapter 4. The rise in ORP value towards the cathode from the anode is a probable result of depleted organic concentration due to microbial activity (Babauta et al., 2012; Tanwar et al., 2008). In the current study also, the mean cathode COD concentration could be correlated with the cathode ORP value during reactor operation. Reactor R'1 showed the minimum effluent COD value and maximum effluent ORP, whereas reactor R2 exerted maximum effluent COD and minimum ORP.

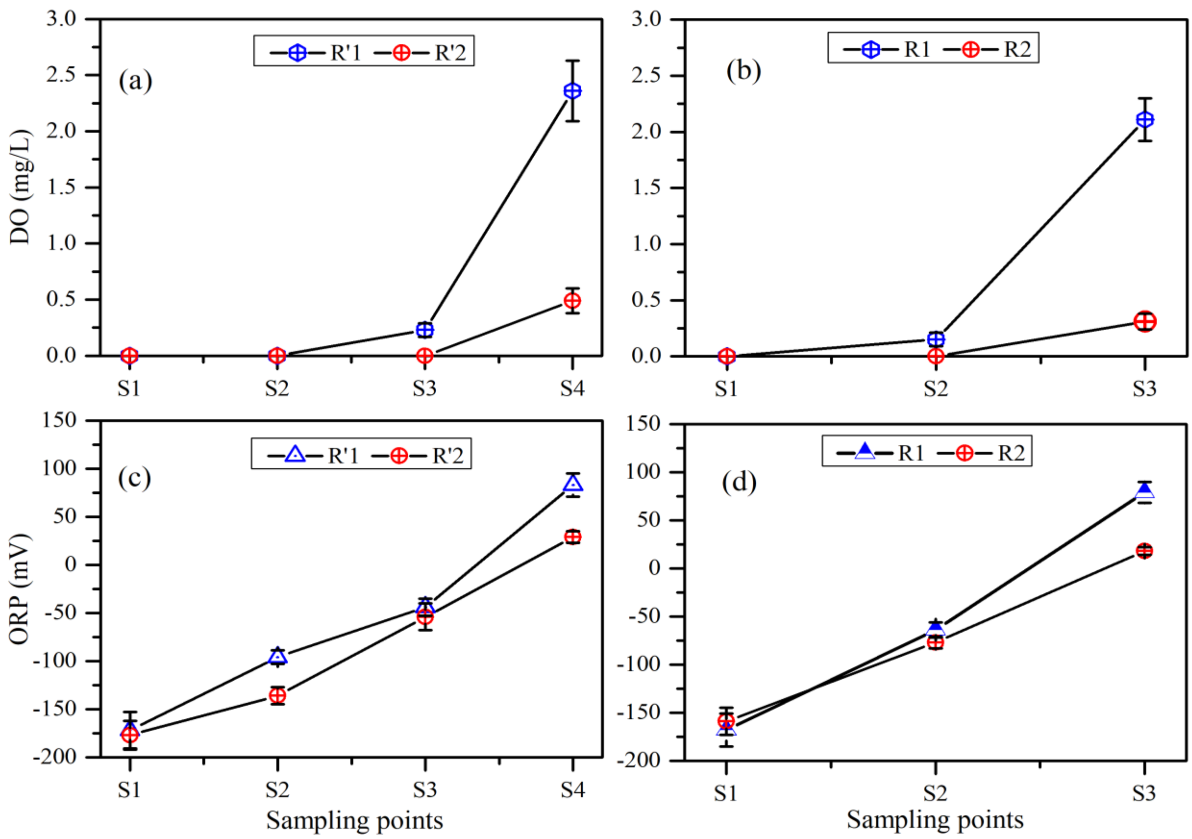

Fig. 6.3 Spatial variation in (a) DO in multi-anode R'1 and R'2, (b) DO in single-anode R1 and R2, (c) ORP in multi-anode R'1 and R'2, (d) ORP in single-anode R1 and R2.

6.3.3 Bioelectricity enhancement in multi-anode CW-MFC

In multi-anode CW-MFC operation, two possible modes of connections are available for completing the external circuit. One is the parallel connection where the cathode is connected to each anode with separate external resistors. The other mode is the combined connection, where the circuit is completed with only one external resistor. In the current study, the cathode was connected to the anodes with a parallel connection as it is more beneficial for extracting higher power output as compared to a combined connection (Tang et al., 2019). In reactors R1 and R2, the mean operating voltages were 749.10±10.90 mV and 475.32±12.12 mV at steady state. The higher voltage output in reactor R1 was obtained due to increased cathode potential as compared to reactor R2 (Fig. 6.3). High cathode potential is a driving factor for enhancing the electrical performance of bioelectrochemical reactors (Bhaduri and Behera, 2024). In the case of multi-anode reactors, separate anodes exerted different operating voltages due to the parallel connection in place. In reactor R'1, the mean operating voltages observed from anodes A1, A2, and A3 at steady state were 736.78±7.42 mV, 791.80±7.12 mV, and 432.66±4.26 mV respectively. In reactor R'2, the mean operating voltages at steady state for anodes A1, A2, and A3 were 464.17±11.32 mV, 488.51±13.07 mV, and 254.95±9.99 mV. The variation in the operating voltages of all reactors throughout the study has been illustrated in Figure 6.4.

The maximum operating voltage was recorded for anode A2 and the minimum operating voltage was recorded for anode A3 in both planted R'1 and unplanted R'2. The output voltage of CW-MFC reactors is generally dependent on the distance between the cathode and the anode. With a reduction in the inter-electrode spacing, the voltage production is supposed to increase (Huang et al., 2021). The internal resistance is also proportional to the distance between electrodes (Doherty et al., 2015). This trend was consistent for anode A1 and A2 in both reactors where the reduced spacing led to a diminution in the internal resistance and a surcharge in the operating voltage. Interestingly the lowest operating voltage was obtained from anode A3, which has the minimum distance from the cathode and has exerted minimum internal resistance. The reason behind this is the limited availability of the soluble organics at anode A3 with a low potential difference as compared to the cathode as seen in Fig 6.2. The spatial COD analysis of R'1 and R'2 revealed that anode A3 had the minimum availability of organic matter to EABs for their metabolic activity as compared to anode A1 and anode A2. This has constrained the production and transfer of external electrons to the anode leading to very low voltage generation (Oon et al., 2016).

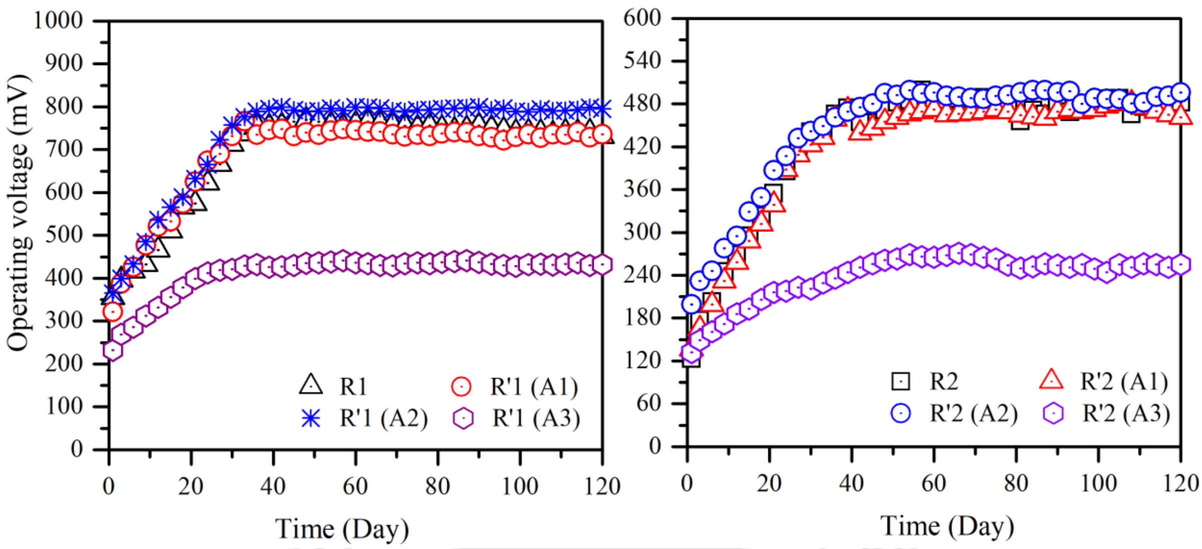

Fig. 6.4 Variation in operating voltage of the reactors corresponding to different anodes during the study.

Table 6.1 summarizes the maximum operating voltage, available COD, inter-electrode spacing, coulombic efficiency, and internal resistances of all the reactors used in the study. Multiple research has been documented in the past analyzing the effect of electrode distance on bioelectricity production using multianode MFCs. However, the applications of multi-anode integrated CW-MFC systems in the literature are few. The bioelectricity production in multi-anode CW-MFC in this study was similar to Oon et al. (2016). The minimum operating voltage was obtained from the anode which was placed nearest to the cathode due to a lack of organics. In another study by Oon et al. (2017), the anode with minimum cathode distance exerted the maximum output voltage under varying external aeration rates. There are other studies also where the anode placed nearest to the cathode has produced the maximum operating voltage in bioelectrochemical systems (Tamta et al., 2023, Oon et al., 2015, Li et al., 2011). It is evident from the findings of this chapter that, the organic availability and the electrode distance both are crucial factors for the resulting operating voltage. Also, it shows that organic availability has a more profound role as compared to anode-cathode distance since operating voltage almost decreased by 50% in A3 (see Table 6.1). Other inherent components such as the electrode potential, packing density, internal resistance, and wetland macrophyte may also contribute to the variation in bioelectricity output. The polarization was performed after 80 days of study when all the reactors were operating at a steady state. In the case of R'1 and R'2 (multi-anode reactors), different power density (PD) vs current density (CD) plots were obtained for different anodes dictating the range of operation of the reactors.

Table 6.1 Organic availability and operating voltage of the reactors corresponding to different anodes with internal resistance and coulombic efficiency

Reactor		Distance from cathode (cm)	Effluent COD (mg/L)	Operating voltage (mV)	Coulombic efficiency (%)	Internal resistance (ohm)
R1-A		20	74.6±6.76	749.10±10.90	0.88	256.85
R2-A		20	98.4±5.45	475.32±12.12	0.68	276.01
R'1	**A1**	20	73.5±6.23	736.78±7.42	0.86	264.59
	A2	15	26.3±3.56	791.80±7.12	2.72	251.63
	A3	10	11.2±2.44	432.66±4.26	4.64	235.67
R'2	**A1**	20	97.3±7.45	464.17±11.32	0.65	276.57
	A2	15	44.8±6.31	488.51±13.07	1.50	263.00
	A3	10	28.6±3.12	254.95±9.99	2.54	193.70

6.3.4 Polarization of reactors, internal resistance and power density

For R1 and R2 reactors, single plots were obtained due to the presence of a single anode. Figure 6.5 (a-d) shows the power density vs. current density plots for reactors R1, R2, R'1, and R'2 respectively. The operating voltage vs. current density plots obtained for reactors R1, R2, R'1, and R'2 are shown in Figure 6.6 (a-d). The internal resistances of the reactors were calculated from the slope of the operating voltage vs current density plots. The maximum PD for reactors R1 and R2 were observed to be 6.05 mW/m^2 and 2.03 mW/m^2. The corresponding CDs for R1 and R2 were 21.54 mA/m^2 and 8.85 mA/m^2. For reactor R'1, the maximum PDs for anode A1, A2, and A3 were 6.05 mW/m^2, 7.47 mW/m^2, and 3.07 mW/m^2. The corresponding CDs were 18.73 mA/m^2, 20.81 mA/m^2, and 13.33 mA/m^2, respectively. The PD and CD values were considerably reduced in R'2 as compared to R'1. The maximum PDs were 1.94 mW/m^2, 2.32 mW/m^2, and 1.59 mW/m^2 for anode A1, A2, and A3. The corresponding CDs were 8.66 mA/m^2, 14.03 mA/m^2, and 7.85 mA/m^2, respectively.

In Fig. 6.5 the peak of the curve shows the maximum output PD that could be achieved from the reactors. The CD corresponding to the peak of the curve shows the optimal range of current density for reactor operation. In the case of single anode reactors (R1 and R2), the peak of the PD curve was higher for the planted reactor probably due to increased operating voltage (Fig. 6.4) that resulted from high cathode ORP. In multi-anode reactors also, the planted CW-MFC (R'1) showed an expanded range of reactor operation as compared to the unplanted CW-MFC. The maximum PD peak was obtained for anode A2, for both R'1 and R'2. The electrode

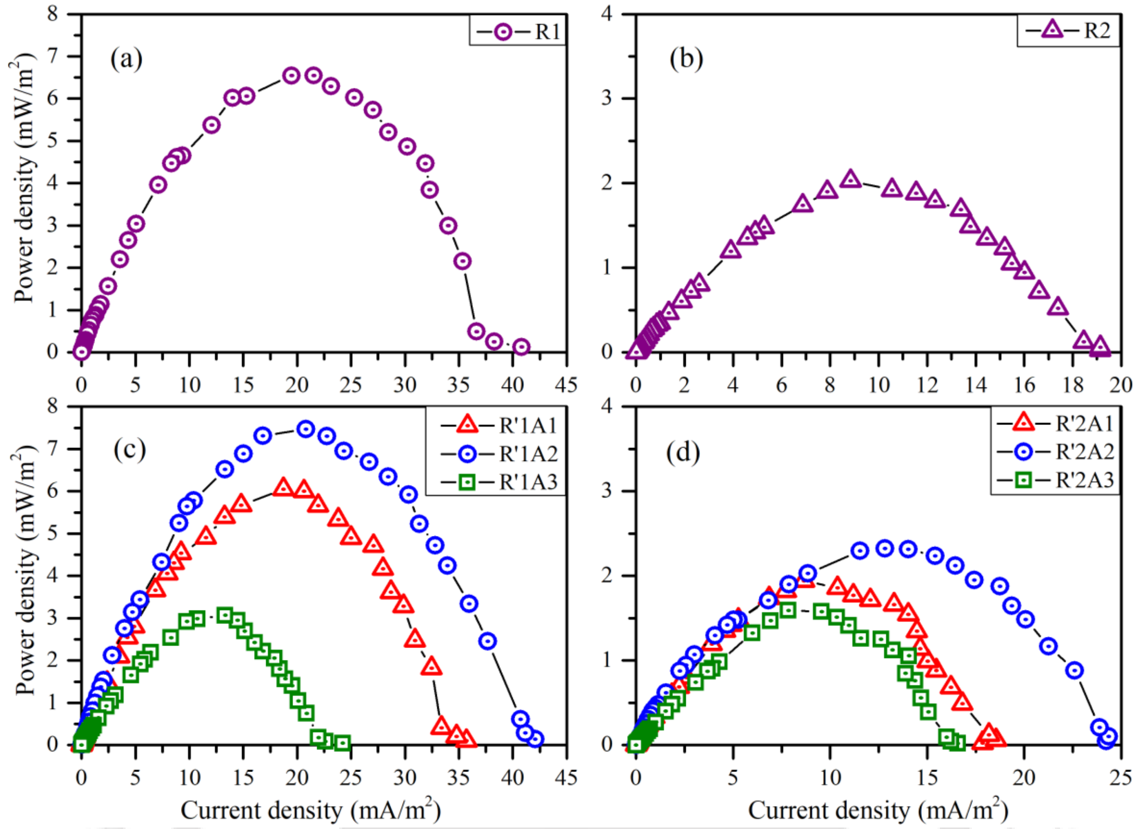

Fig. 6.5 Power density vs. current density plots of different reactors obtained during the study

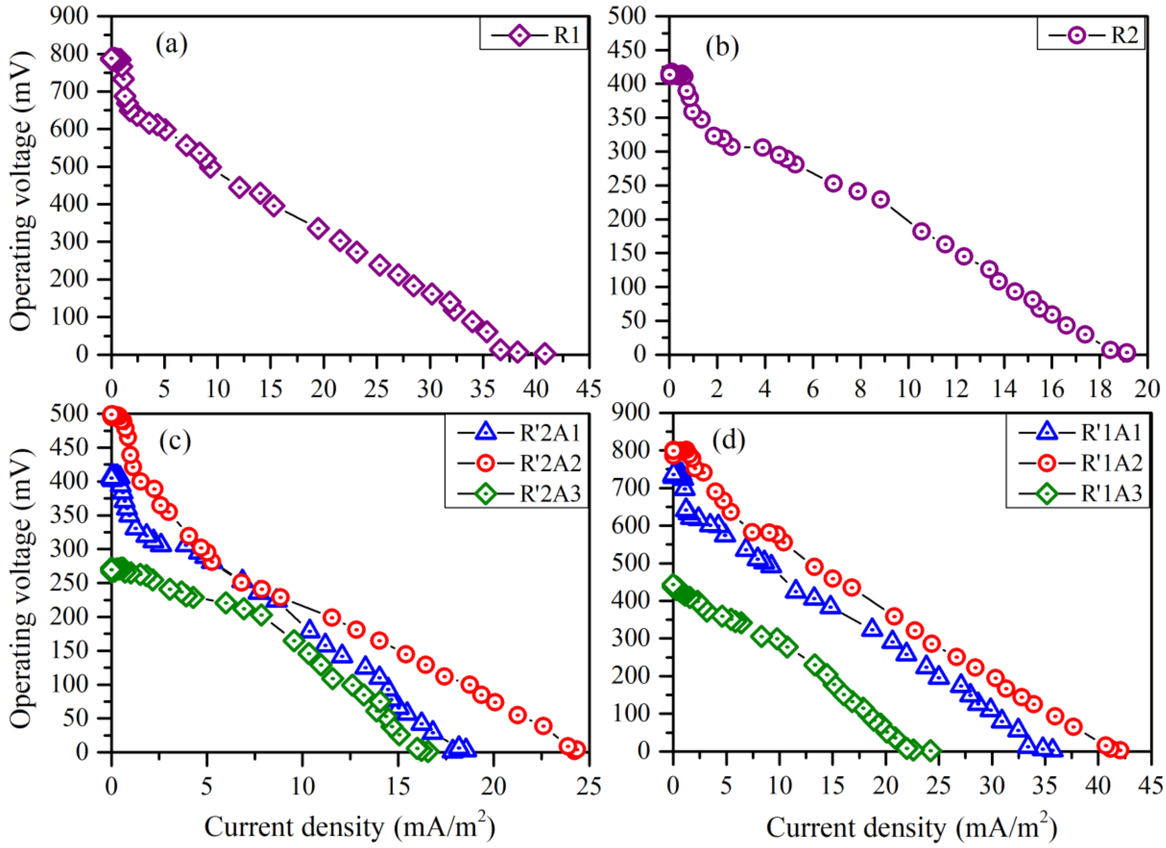

Fig. 6.6 Polarization plots obtained for different reactors during the study

distance for anode A2 was lower than for anode A1 which led to reduced internal resistance. For anode A3 the peak was reduced significantly due to lower organic availability as reported in Table 6.1. Generally, the polarization response of different bioelectrochemical reactors depends on multiple factors like packing density, internal resistances, internal losses, electrode materials, etc. The application of multi-anode reactors produced better wastewater treatment and bioelectrical performance as compared to single-anode reactors. Due to an increase in the effective anode surface area, the algebraic sum of the mean operating voltages was increased to approximately 1961 mV in R'1 from 749 mV in R1. For reactor R'2, the sum of operating voltages obtained from three anodes was 1207 mV as compared to 475 mV in R2.

The internal resistances in the current study varied with the interelectrode distance. With an increase in the electrode spacing, the internal resistance increased. For multi-anode reactors (R'1 and R'2), an increase in the power output was observed from anode A1 to A2 with a simultaneous reduction in the internal resistance as visible in Table 6.1. However, the power output in anode A1 was lower compared to anode A2 and anode A3. This suggests that the internal resistance was not the critical factor that was affecting the power output of the multi-anode reactors. The organic availability and the difference in redox potential also played crucial roles. However, the coulombic efficiency was observed to increase with a reduction in the internal resistance in all the reactors. The application of multi-anode reactors has produced higher coulombic efficiency as compared to single-anode reactors in both planted and unplanted configurations. Table 6.2 outlines the performance comparison of the multi-anode CW-MFC (R'1) used in the current study with previous pieces of literature that have employed multi-anode CW-MFCs for wastewater treatment.

6.3.5 Biomass activity study during open and closed circuit operation

The heterotrophic biomass activity was estimated for all the reactors twice during the study. The first analysis was performed during closed-circuit operation after 90 days. The second analysis was performed after 120 days during open circuit operation. The objective was to analyze the changes in the overall COD removal efficiencies of the reactors due to the changes in circuit connection modes (closed-circuit to open-circuit). The specific heterotrophic activities are summarized in Table 6.3. The activity was determined from the maximum slope of the cumulative COD removal assay which corresponds to the maximum substrate utilization by the reactor biomass. The biomass activity of different reactors in terms of cumulative COD removal over time, for the closed-circuit and open-circuit operation has been illustrated in

Figure 6.7 and Figure 6.8 respectively. A reduction in the specific heterotrophic activity was observed during open-circuit operation in all the reactors as compared to closed-circuit operation.

During closed-circuit operation, the maximum biomass activity was observed in reactor R’1 (planted multi-anode CW-MFC). In the case of unplanted reactors, R’2 (multi-anode) showed higher biomass activity as compared to R2 (single-anode). It could be inferred from the activity study that the presence of additional electrodes as electron acceptors (anode) led to an increase in the flow of electrons (performed by electroactive bacteria) which resulted in higher COD depletion in multi-anode reactors. During open-circuit operation, the flow via the external circuit was hampered which reduced the COD removal efficiency of the reactors, and a similar trend was portrayed by the activity study results. Interestingly, reactor R1 and R2 during closed circuit operation showed comparatively similar activity obtained in Chapter 3 (section 3.3.4), exerted by the planted CW-MFC reactor R2 (1.27 mg COD mg VSS^{-1} d^{-1}) and unplanted CW-MFC reactor R3 (0.93 mg COD mg VSS^{-1} d^{-1}). This was probably due to the similar reactor configuration, the same source of feed wastewater during reactor operation, and the identical carbon source (acetate) used for the batch activity study.

6.3.6 Fecal coliform and *E. coli* inactivation from domestic wastewater

The performance of all the reactors was assessed in terms of the fecal coliform (FC) and *Escherichia coli* (*E. coli*) removal during the treatment of domestic wastewater. *E. coli*, is a well-known indicator organism that shows an electroactive nature. Multiple researchers have previously applied *E. coli* for the inoculation of bioelectrochemical reactors like MFCs (Nasirahmadi and Safekordi, 2011; Sugnaux et al., 2013). The current study was undertaken to appraise the comparative inactivation of *E. coli* and FC present in a mixed microbial population under different circuit conditions in single and multi-anode integrated CW-MFC systems. In the domestic wastewater, the mean concentration of FC and *E. coli* was (7.13±1.55) E+06 and (9.75±2.56) E+06, respectively as MPN/100 mL. During closed-circuit operation, the maximum FC removal was obtained from reactor R’1. The mean FC removal efficiencies of reactors R1 and R’1 were log 3.89±0.22 and log 4.23±0.20. In the unplanted reactors, multi-anode R’2 performed better FC inactivation as compared to the single-anode reactor R2. The mean FC removal efficiencies of R2 and R’2 were log 3.59±0.19 and log 3.87±0.24 respectively. This shows that the incorporation of multi-anode assembly in CW-MFC reactors (planted or unplanted) could be beneficial for higher FC inactivation.

Table 6.2 Comparative literature summary of multi-anode CW-MFC and MFC reactors with the current study

Reactor type and number of anodes	Reactor volume and HRT	Wastewater details	Electrode details	Collective PD and operating voltage	Reference
CW-MFC planted with *Typha angustifolia* No. of anodes: 3	Eff. vol. 4.6 L HRT: 36 h	Domestic wastewater. COD: 212 mg/L pH: 7.06	Anode: SSM+GAC+graphite rod Cathode: GAC+graphite rod	Max PD: 16.6 mW/m^2 Max OV: 1960 mV COD removal: 97%	Current study
CW-MFC (unplanted) No. of anodes: 3	Eff. vol. 4.6 L HRT: 36 h	Domestic wastewater. COD: 212 mg/L pH: 7.06	Anode: SSM+GAC+graphite rod Cathode: GAC+graphite rod	Max PD: 5.85 mW/m^2 Max OV: 1206 mV COD removal: 91.6%	Current study
CW-MFC (unplanted) No. of anodes: 4	Eff. vol. 1.7 L HRT: 24 h	Synthetic household wastewater. Glucose: 1000 mg/L $(NH)_2SO_4$: 11.19 mg/L	Anode: Granular activated charcoal Cathode: Granular activated charcoal	Max PD: 48.3 mW/m^3 Max OV: 262 mV COD removal: 95%	Tamta et al. (2023)
MFC (unplanted) Tubular configuration No. of anodes: 6	Eff. vol. 50 L HRT: 48 h	Primary sedimentation tank effluent. COD: 168 mg/L BOD: 76 mg/L NH_3: 35 mg/L	Anode: Carbon felt bunch anodes Cathode: Carbon-based cathode with SSM mesh	Max PD: 25 mW/m^2 Max OV: 400 mV COD removal: 65%	Xie et al. (2023)
MFC (unplanted) No. of anodes: 6	Eff. vol. 1.5 L HRT: 300 h	Sewage, slaughterhouse, and hospital wastewater COD: 1246, 1951, and 1114 mg/L TSS: 602, 1016, 984 mg/L TN: 40.7, 384, 412 mg/L	Anode: Carbon plate Cathode: Carbon plate	Max PD: 2599 mW/m^2 Max OV: 675 mV COD removal: 62.7%	Opoku et al. (2022)

Table 6.2 continued…

CW-MFC (unplanted) No.of anodes: 4	Eff. vol. 30 L HRT: 36 h	Synthetic domestic wastewater COD: 500 mg/L NH_3: 30 mg/L	Anode: SSM Cathode: Carbon felt	Max PD: 17.5 mW/m^2 Max OV: 2300 mV COD removal: 91.7%	Tang et al. (2019)
CW-MFC planted with *Elodea nutallii* No.of anodes: 3	Total vol. 19 L HRT: 24 h	Synthetic domestic wastewater COD: 648 mg/L NH_3: 41 mg/L	Anode: GAC Cathode: GAC	Max PD: 184 mW/m^3 Max OV: 1470 mV COD removal: 99%	Oon et al. (2017)
CW-MFC planted with *Typha latifolia* No.of anodes: 3	Total vol. 19 L HRT: 24 h	Synthetic domestic wastewater COD: 314 mg/L NH_3: 40 mg/L	Anode: Carbon felt Cathode: Carbon felt	Max PD: 12 mW/m^3 Max OV: 950 mV COD removal: 100%	Oon et al. (2015)
CW-MFC planted with *Typha latifolia* No.of anodes: 3	Total vol. 19 L HRT: 24 h	Synthetic domestic wastewater COD: 624 mg/L NH_3: 40 mg/L	Anode: GAC Cathode: GAC	Max PD: 189 mW/m^3 Max OV: 972 mV COD removal: 99%	Oon et al. (2015)
MFC (unplanted) No. of anodes: 3	Total vol. 130 mL HRT: 16 h	Domestic wastewater COD: 275 mg/L BOD_5: 111 mg/L Conductivity: 1.4 mS/cm	Anode: Graphite fiber brush Cathode: Carbon cloth	Max PD: 120 mW/m^3 Max OV: 700 mV COD removal: 90%	Ahn and Logan (2013)

PD: Power density, SSM: Stainless steel mesh, GAC: Granular activated carbon. OV: Operating voltage, Eff. vol.: Effective volume. N.B: The collective performance of the reactors was determined using the sum of the PD and OV of individual anodes of the reactors.

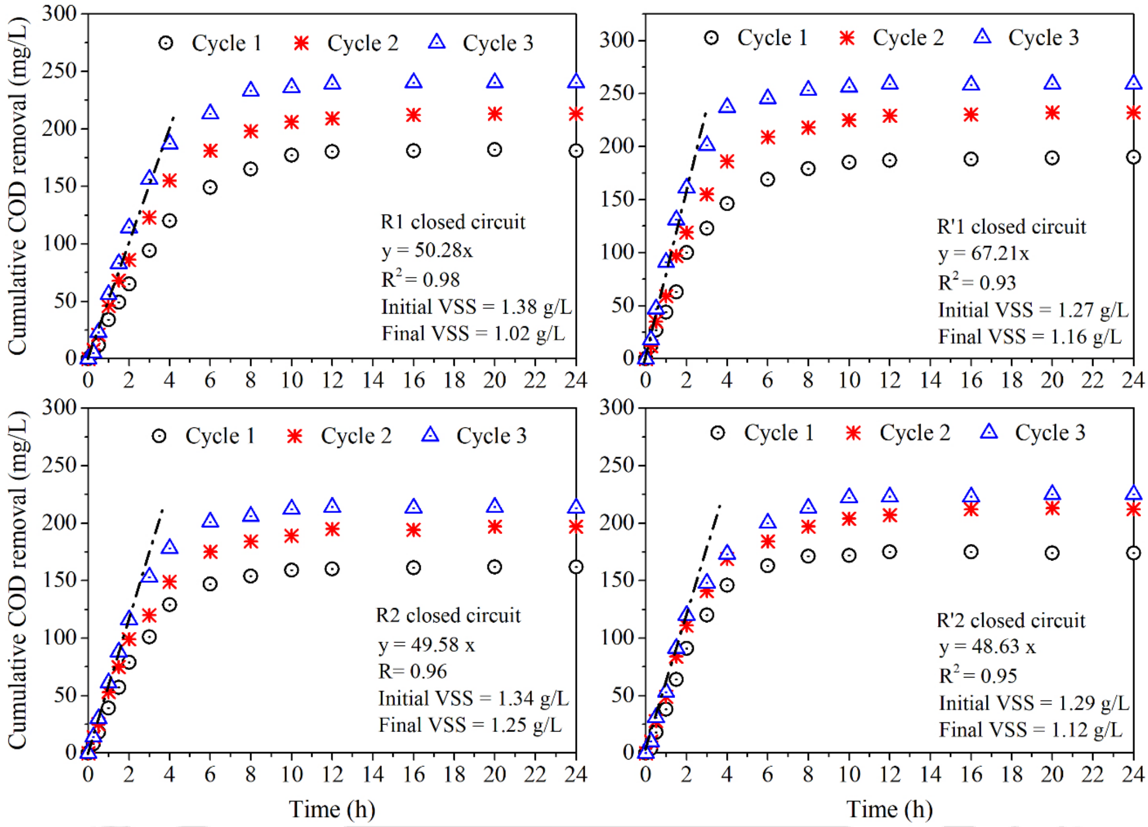

Fig. 6.7 Cumulative COD removal by the reactor biomass during closed-circuit operation

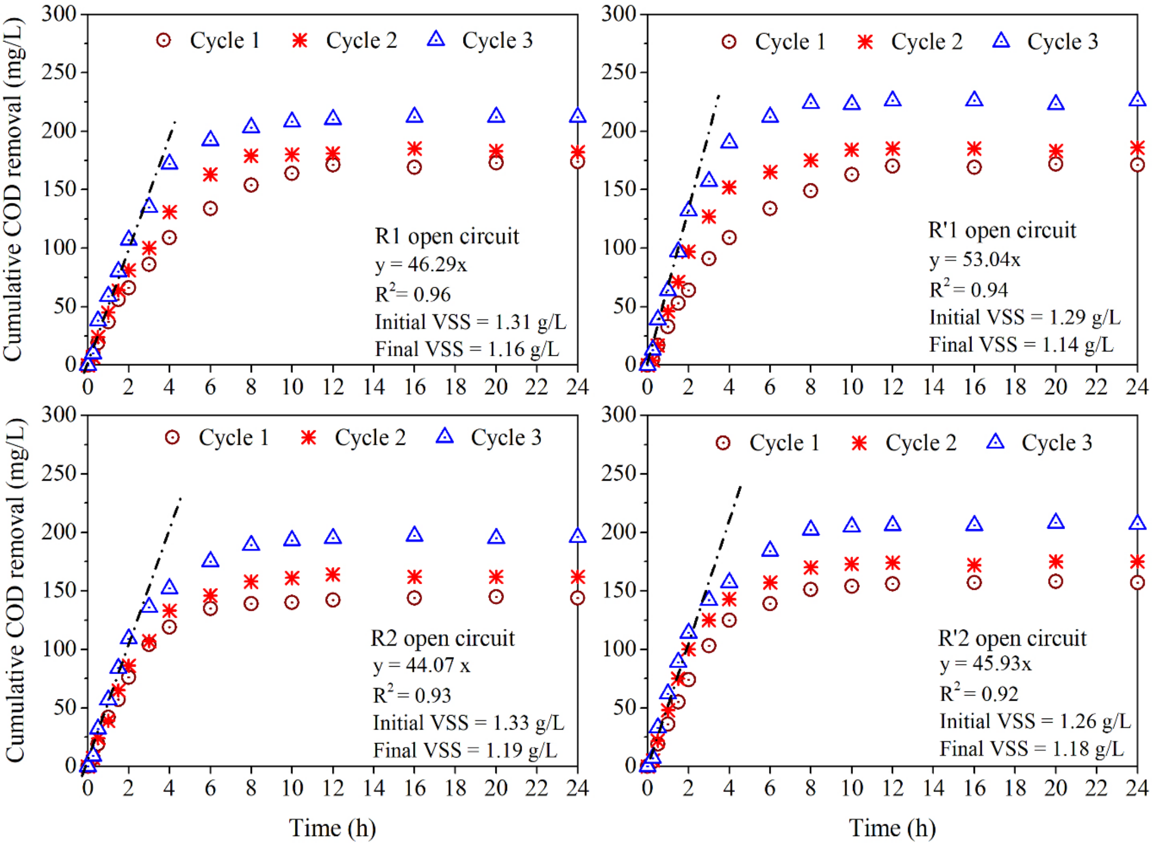

Fig. 6.8 Cumulative COD removal by the reactor biomass during open-circuit operation

Table 6.3 Biomass activity of the reactors during open and closed-circuit operation

Biomass activity (mg COD removed mg VSS^{-1} d^{-1})	**Reactor R1**	**Reactor R'1**	**Reactor R2**	**Reactor R'2**
Closed circuit mode	1.24	1.39	0.95	0.96
Open circuit mode	0.96	1.12	0.89	0.93

When the external circuits were disconnected, a considerable reduction in the FC removal efficiency was observed in all the reactors, portraying the importance of the electron flow to the cathode which are generated via EAB activity at the anodes. During open-circuit, the FC removal in reactors R1, R'1, R2, and R'2 were log 3.45±0.12, log 3.49±0.15, log 3.13±0.16, and log 3.22±0.23, respectively.

Interesting results were obtained in the course of the assessment of *E. coli* inactivation. During closed-circuit operation, not much difference was observed between the *E. coli* removal efficiencies of reactor R1 and R'1. Reactor R1 showed a mean removal of log 3.29±0.12 and reactor R'1 exhibited a mean removal efficiency of log 3.16±0.13. In the case of unplanted reactors, R2 and R'2 showed mean *E. coli* inactivation efficiencies of log 2.79±0.13 and log 2.85±0.24. Increasing the number of electrodes did not show much effect on *E. coli* elimination from the wastewater. When the reactors were operated in open-circuit condition, the mean *E. coli* removal in reactors R1, R'1, R2, and R'2 was 3.22±0.22, 3.31±0.19, 2.74±0.15, and 2.67±0.19. The *E. coli* removal efficiency of individual reactors in open-circuit operation was close to the efficiencies observed during closed-circuit mode. When compared to the FC removal performance, the *E. coli* removal in all the CW-MFC reactors reduced considerably. One-way ANOVA analysis revealed a significant difference between the FC and E coli removal efficiencies of individual reactors ($p < 0.05$). Figure 6.9 shows the FC and *E. coli* removal efficiency in the reactors with time for the whole study period. From Figure 6.9, a drop in the efficiency is well visible at the start of open-circuit operation. At the end of the study, (120 days) *E. coli* removal from all reactors became almost similar to FC removal.

In the current study, planted reactors (R1 and R'1) showed better FC and *E. coli* removal performance, as compared to the unplanted reactors (R2 and R'2). The presence of macrophytes leads to better removal of coliform organisms due to factors like adsorption and filtration by the surface of the plant roots and the bactericidal properties of the root exudates, etc. (Rivera et al., 1999; Wu et al., 2016; Chand et al., 2021). A similar scenario might have

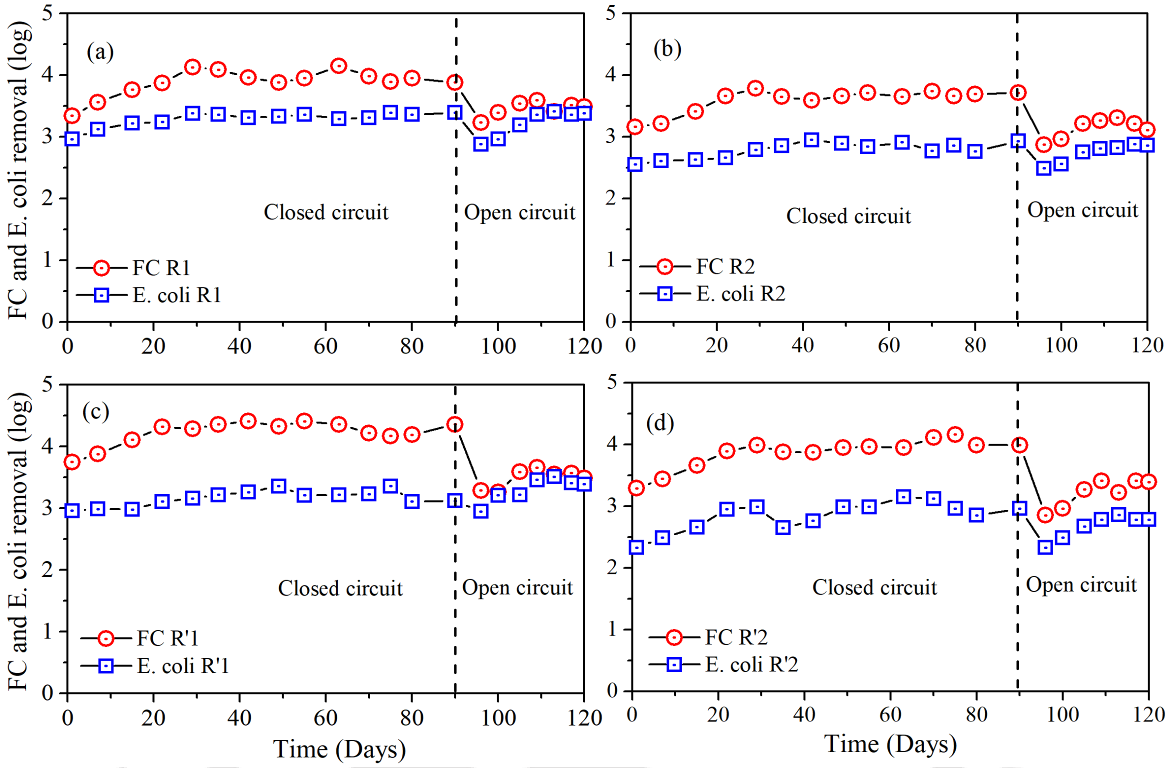

Fig. 6.9 Variation in FC and *E. coli* removal efficiency of (a) reactor R1, (b) reactor R2, (c) reactor R'1, and (d) reactor R'2 during the study period

also prevailed in the current study which led to better *E. coli* and FC inactivation in the reactors planted with *Typha angustifolia*, as compared to the unplanted reactors. In bioelectrochemical systems like MFCs, fecal pathogens could be inactivated by the processes related to bioelectricity generation. It was hypothesized by Ieropoulos et al. (2019), and Ieropoulos et al. (2017), that exogenous pathogenic microbes (non-EABs) may be outcompeted by the established population of active EABs, capable of performing external electron transfer during organic degradation.

The current research showed that the FC removal efficiency was higher than the *E. coli* removal in the CW-MFC reactors during closed-circuit operation. When the reactors were shifted to open-circuit mode, the FC removal decreased, and the difference between the *E. coli*, and the FC removal reduced as seen in Figure 6.8. This may be attributed to the electroactive nature of *E. coli*. Some *E. coli* strains are capable of performing external electron transfer similar to that of established EABs like *Geobacter* (Castellano-Hinojosa et al., 2022; Logan et al., 2019). When the circuit was connected, electroactive *E. coli* strains were probably capable of the electron transfer which resulted in less inactivation inside the reactors. When the external circuit was disconnected, electroactive *E. coli* strains were no longer able to perform the electron transfer due to which a subtle rise in *E. coli* removal efficiency could be observed during 90 – 120 days of reactor operation.

6.4 Conclusion

The use of multiple anodes provided a larger surface area for the EABs to attach and generate a higher amount of extracellular electron flux. This led to a surcharge in the total operating voltage and power density output (as cumulative of individual anodes) in the multi-anode system compared to the single-anode system. *Typha angustifolia* played an important role by increasing the cathode redox potential through radial oxygen loss and contributed to the enhanced bioelectrical performance of the planted reactors. In the case of multi-anode CW-MFCs, the voltage and power density of individual anodes varied with the distance from the cathode. The optimum performance was obtained from the intermediate anode where both available COD and the distance from the cathode were key factors. The nearest anode from the cathode produced the least operating voltage and power density due to the very low amount of available COD. The wastewater treatment performance of the reactors (both single and multi-anode CW-MFCs) in terms of COD and FC removal was better during the closed-circuit operation. When the circuits were opened, COD and FC inactivation in all the reactors were reduced significantly. This shows the importance of closed-circuit electron flow for promoting a desirable level of organic degradation and fecal pathogen inactivation in integrated CW-MFC systems. The inactivation efficiency of *E. coli* was found to be lower than the FC removal in all the reactors. The electro-active nature of *E.coli* probably helped them survive and compete with other EABs during closed-circuit operation of the reactors. In the open-circuit mode, the *E. coli* inactivation efficiency showed a slight rising trend, and the difference between FC and *E. coli* removal efficiencies of individual reactors diminished. Planted CW-MFCs showed better removal for FC and *E. coli* as compared to the unplanted reactors which could be attributed to the mechanisms like filtration and adsorption of organisms by the rhizome network of *Typha angustifolia*. In the future, a comparative assessment of the microbial population between single-anode and multi-anode CW-MFC reactors could be done to investigate the effect of multiple anodes on the diversity and dominance of different EAB populations.

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Chapter 7

Conclusion and Future Scope

This chapter is comprised of the major findings that were obtained from the research. During the research work, certain limitations were observed which are worthy of mentioning in this chapter. The chapter also includes some recommendations for the future, based on the experiences of the current research.

7.1 Major findings from the research

This research was primarily aimed at the bioelectrochemical treatment of domestic wastewater employing a lab-scale integrated constructed wetland-microbial fuel cell (CW-MFC) reactor, prioritizing the removal of organic contaminants and fecal coliforms (FC). The seasonal performance variation of the reactor was monitored and the aspect of bioelectricity generation during the treatment of wastewater was assessed. The mechanisms related to the inactivation of FC inside the integrated CW-MFC were investigated. The microbial population associated with the integrated CW-MFC system was assessed using microbial metagenomics. Performance assessment of the CW-MFC system was done under varying HRTs for long-term treatment and the roles of the reactor media and the wetland macrophyte (*Typha angustifolia*) were examined. Cost-benefit analysis of the integrated CW-MFC system was conducted to check the economic benefits in terms of achievable revenues from wastewater treatment, bioelectricity production, and areal footprint reduction. Multi-anode CW-MFC reactor was employed for the enhanced FC inactivation and bioenergy conversion and a comparative performance assessment was done with a single-anode CW-MFC reactor. The major outcomes of the research are highlighted below:

1. The planted CW-MFC reactor (R2) was highly efficient in COD degradation and FC inactivation as compared to the other two reactors R1 (constructed wetland) and R3 (unplanted CW-MFC). FC inactivation performance was observed to be affected by seasonal temperature fluctuations. Due to temperature reduction in winter, the rate of FC inactivation dropped considerably.

2. Metagenomic analysis of the reactor biomass showed more dominance and diversity of EABs (*Firmicutes*, *Bacteroidetes*, *Actinobacteria*, etc.) in reactor R2. The presence of the aerobic *Nitrospira* in the planted reactors R1 and R2 indicated the possible development of a micro-aerobic environment near the rhizosphere of *Typha*.

3. During batch mode reactor operation, the maximum FC inactivation was observed at the anode of R2 with a closed circuit. This highlights the importance of EAB activity and external electron transfer to the anode and the subsequent flow of the extracellular electrons to the cathode via the external circuit.

4. The performance of the integrated CW-MFC reactor in terms of organics and FC removal and bioelectricity generation deteriorated with the stepwise reduction in the HRT. HRTs of 36 h and 24 h were rendered optimal for satisfactory FC removal. The best performance of the CW-MFC was obtained from an HRT of 36 h in terms of combined wastewater treatment with bioelectricity production.

5. The contribution of *Typha angustifolia* was crucial for increasing the dissolved oxygen at the cathode of R2. This led to increased redox potential at the cathode of R2 and higher bioelectricity generation as compared to unplanted R3. The presence of *Typha* diversified the bacterial population in the planted reactors (R1 and R2). During the cost-benefit analysis, the planted reactor R1 (without electrodes) showed better economic benefits than reactor R3 associated with electrodes (without plants).

6. The cost-benefit analysis established the CW-MFC as the most economical solution for domestic wastewater management offering revenue from bioelectricity generation and wastewater treatment. A major source of revenue was obtained from the reuse of treated wastewater and the integrated CW-MFC system was capable of acquiring this benefit without the application of the disinfection process.

7. Integrated CW-MFC was capable of a significant diminution in the areal footprint (76% reduction) of the reactor without effluent disinfection producing similar effluent quality as compared to the standalone constructed wetland.

8. The application of multi-anode CW-MFC boosted overall coulombic efficiency and voltage output as compared to single-anode reactors. This shows the prospects of multi-anode systems for achieving better bioenergy conversion from low-strength domestic wastewater. The *E. coli* removal in the multi-anode system was less than the FC inactivation. This is possibly due to the electroactive nature of *E. coli* that allows them to interact with the anode via external electron transfer.

7.2 Limitations and future scope of the current research

The major limitations that were experienced from the research are listed below:

1. The current research was conducted with low-strength domestic wastewater with a lab-scale integrated CW-MFC reactor. Future studies may explore the treatment of medium and high-strength domestic wastewater with an application of pilot-scale reactors.
2. In the current research, the reactors were inoculated with raw domestic wastewater having a diverse microbial population. Reactors inoculated with pure EAB strains (*Firmicutes*, *Bacteroidetes*, etc.) can be employed in the future to check the FC and *E. coli* inactivation under different reactor operational strategies.
3. This research was primarily concentrated on organics and FC inactivation for reuse in irrigation purposes, and the prospect of nutrient removal from the wastewater was not investigated. Future studies may focus on the aspects of nutrient removal and recovery.
4. Innovative operational strategies could be adopted to increase the cathode ORP without artificial aeration, for example, partial effluent recirculation or tidal flow operation. Future studies may also focus on the remediation of emerging contaminants from domestic wastewater by applying integrated CW-MFC reactors.
5. It is worth mentioning that the outcome of the cost-benefit analysis may vary during the installation and operation of field-scale reactors as scaling up bioelectrochemical systems exerts a nonlinear response corresponding to lab-scale reactors.
6. The cost-benefit analysis was performed by adopting a flat rate of benefit from wastewater reuse for irrigation (₹ 5.19/m^3) and for the cost of disinfection (₹ 76/m^3). However, in real scenarios, these rates may vary significantly depending on the designated use of treated wastewater and the amount of chlorine dose required for disinfection.
7. The current study was focused on the inactivation of indicator organisms (TC, FC, and *E. coli*) in a CW-MFC reactor. Future research may investigate the fate of other pathogenic organisms like *Salmonella, Giardia,* etc.

Appendix – A

Table A1 Single-factor ANOVA analysis for effluent BOD_5 of R1 and R2

Summary

Groups	Count	Sum	Average	Variance
R1	32	262.45	8.201563	1.0904
R2	32	232.2	7.256250	12.61802

ANOVA

Source of variation	SS	df	MS	F	*P* value	F crit
Between groups	14.297	1	14.297	1.032	0.313	3.995
Within groups	858.961	62	13.854			
Total	873.259	63				

Table A2 Single-factor ANOVA analysis for effluent BOD_5 of R2 and R3

Summary

Groups	Count	Sum	Average	Variance
R2	32	232.2	7.256	12.618
R3	32	251.47	7.858	14.950

ANOVA

Source of variation	SS	df	MS	F	*P* value	F crit
Between groups	5.802	1	5.802	0.420	0.518	3.995
Within groups	854.614	62	13.784			
Total	860.416	63				

Table A3 Single-factor ANOVA analysis for effluent COD of R1 and R2

Summary

Groups	Count	Sum	Average	Variance
R1	68	1487.95	21.881	78.290
R2	68	807.72	11.878	79.295

ANOVA

Source of variation	SS	df	MS	F	*P* value	F crit
Between groups	3402.301	1	3402.3	43.18	1.02E-09	3.991
Within groups	10558.295	134	78.793			
Total	13960.595	135				

Table A4 Single-factor ANOVA analysis for effluent COD of R2 and R3

Summary

Groups	Count	Sum	Average	Variance
R2	68	807.72	11.878	79.295
R3	68	1578.208	23.208	59.148

ANOVA

Source of variation	SS	df	MS	F	*P* value	F crit
Between groups	4365.086	1	4365.086	63.059	7.19E-13	3.911
Within groups	9275.767	134	69.222			
Total	13640.853	135				

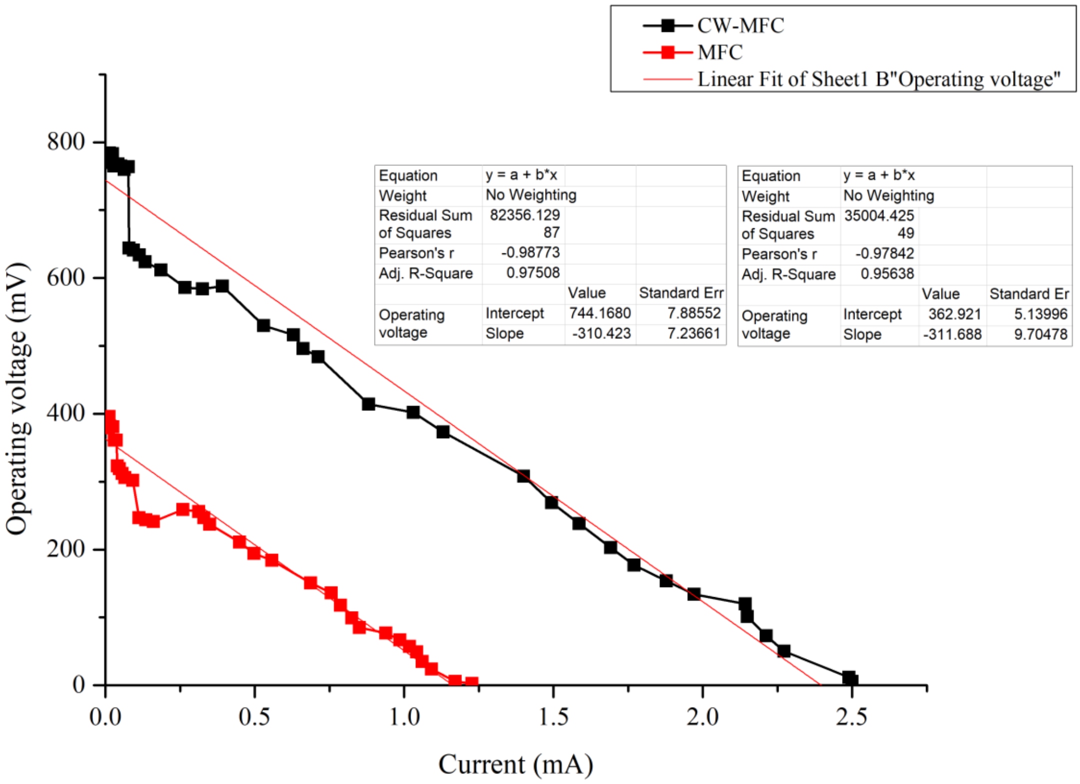

Fig. A1 Analysis of polarization plot of R1 (MFC) and R2 (CW-MFC)

Appendix – B

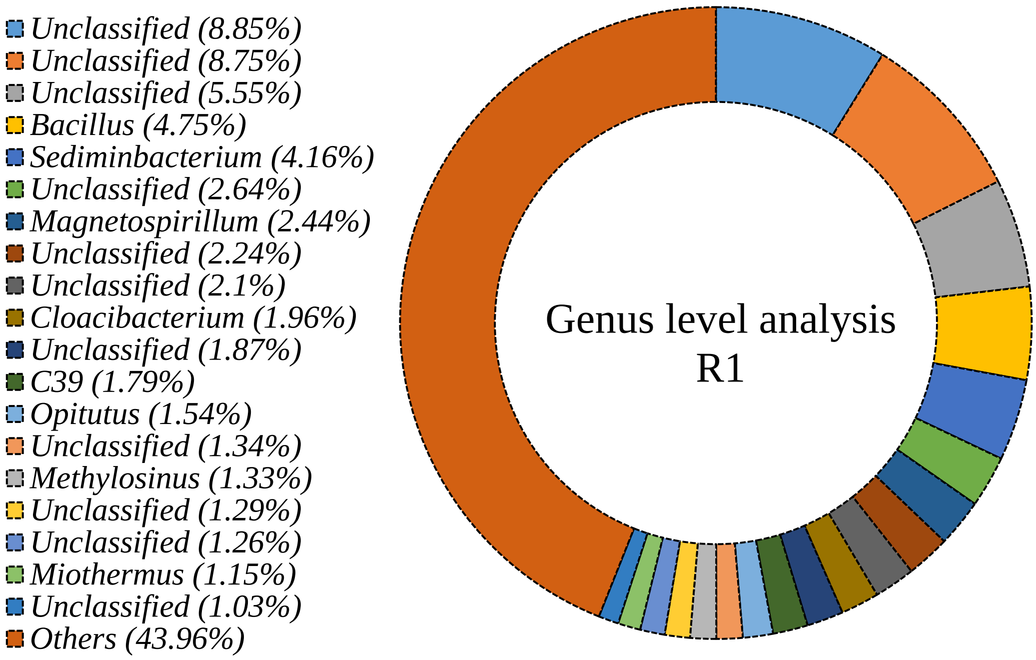

Fig. B1 Relative abundance (%) of microbial lineages at genus level in Reactor R1

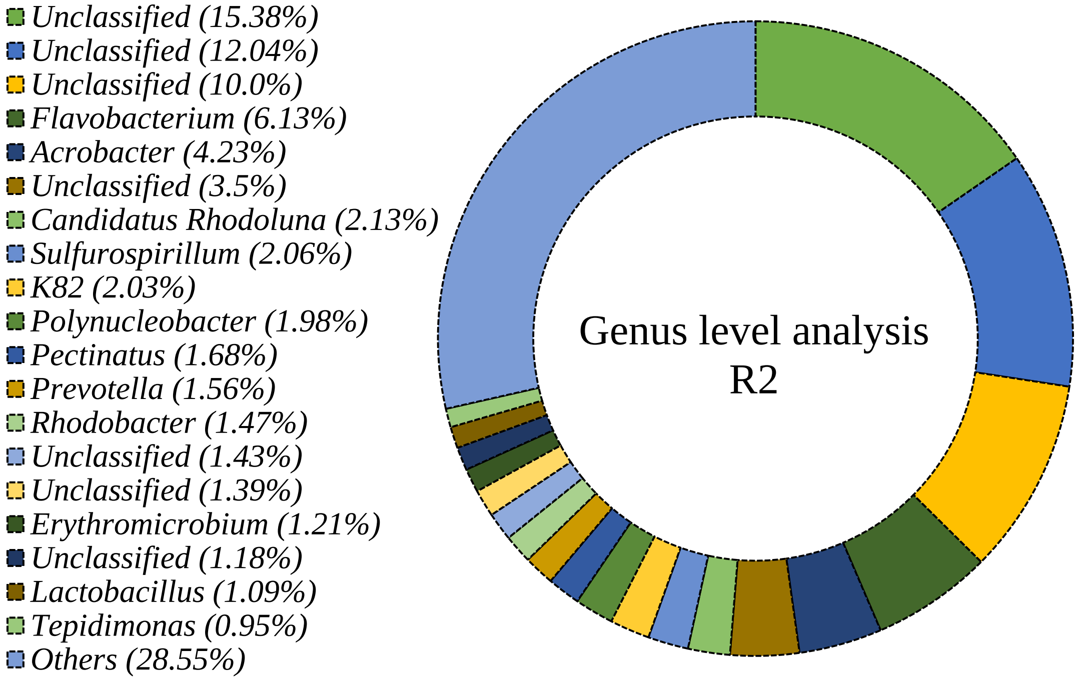

Fig. B2 Relative abundance (%) of microbial lineages at genus level in Reactor R2

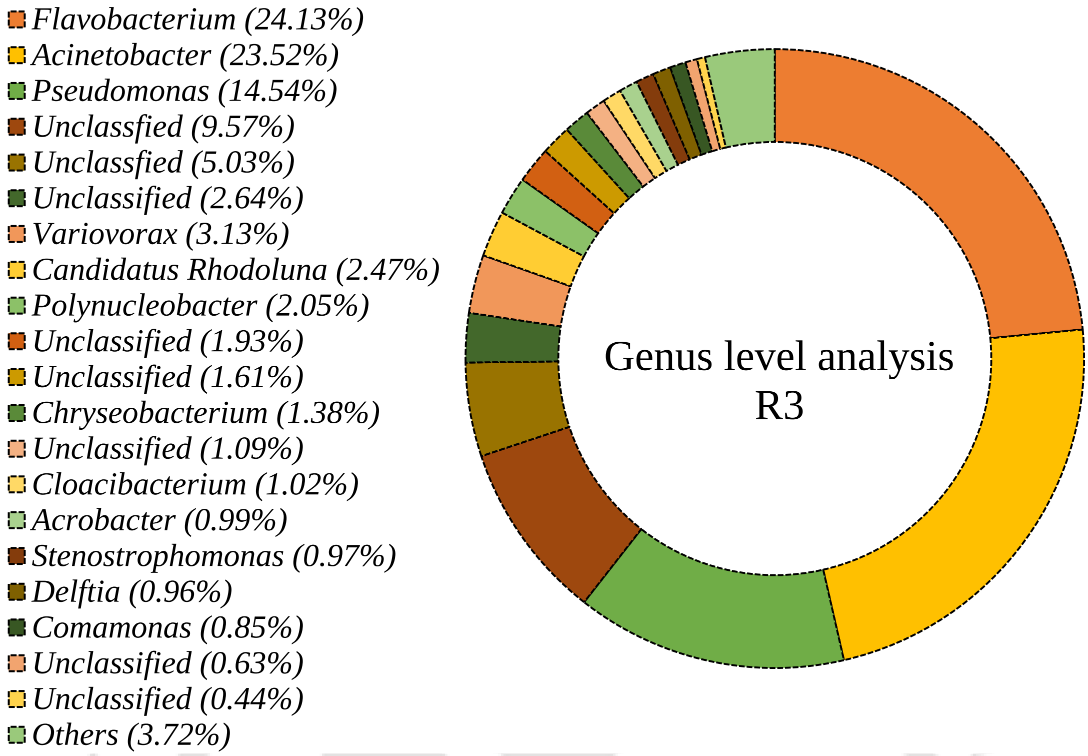

Fig. B3 Relative abundance (%) of microbial lineages at genus level in Reactor R3

B1. Microbial metagenomics details

(a) Genomic DNA extraction, amplification, library preparation and sequencing

Metagenomic DNA was isolated using the commercially available kit (Nucleospin Soil, Macherey-Nagel) and the quality of the isolated metagenomic DNA sample was quantified using a NanoDrop spectrophotometer (NanoDrop One, ThermoScientific). The first amplicon polymerase chain reaction (PCR) was set up using the isolated metagenomic DNA along with a bacterial 16S V3–V4 region-specific primer set. Forward and reverse primers used for the amplification of 16S rRNA targeting bacteria were GCCTACGGGNGGCWGCAG and ACTACHVGGGTATCTAATCC, respectively. The PCR product (3µL) was resolved on 1.2% agarose gel at 120V for approximately 60 minutes (or till the sample reached 3/4th of the gel). The first amplicon generation was followed by next-generation sequencing (NGS) library preparation using Nextera XT Index Kit (Illumina Inc.) (Illumina, 2013). The library was sequenced on the MiSeq platform using 2×300 bp chemistry. The amplicon with the Illumina adaptor was amplified using i5 and i7 primers that added multiplexing index sequences as well as common adapters required for cluster generation (P5 and P7). The amplicon library was purified by AMPure XP beads and quantified using a Qubit Fluorometer (Qubit 3.0 Fluorometer, Life Technology). The amplified library was analyzed on a 4200 Tape Station system (Agilent Technologies, USA) using D1000 Screen tape as per manufacturer instructions. After obtaining the mean peak sizes from the tape station profile, the library was loaded onto MiSeq at an appropriate concentration (10–20 pM) for cluster generation and sequencing. The kit reagents (MiSeq Reagent Kit v3, Illumina) were used in the binding of samples to complementary adapter oligos on paired-end flow cells. The adapters were designed to allow selective cleavage of the forward strands after re-synthesis of the reverse strand during sequencing. The copied reverse strand was then used to sequence from the opposite end of the fragment.

(b) Sequence data analysis

Quantitative Insights into Microbial Ecology (QIIME) pipeline (v1.8) was used to process raw reads. High-quality clean pair end (PE) reads were obtained using Trimmomatic (v0.38) and then stitching of PE data into single-end reads. Operational taxonomic units (OTUs) were obtained by comparing sequence similarity to the Greengenes database (version 13_8) (McDonald et al., 2012). All the sequences from the samples were clustered into Operational Taxonomic Units (OTUs) based on their sequence similarity using the UCLUST algorithm at 97% sequence similarity.

B2. Submission of metagenomics data to NCBI

❖ The genome sequencing data of R1 and R2 reactors have been deposited to the National Centre for Biotechnology Information (NCBI), with the following details:
Bioproject id: PRJNA820999
Biosample accession (R1): SAMN27035155
Biosample accession (R2): SAMN27035156

❖ The genome sequencing data of R1 and R2 reactors have been deposited to the National Centre for Biotechnology Information (NCBI), with the following details:
Bioproject id: PRJNA984182
Biosample accession (R3): SAMN35742750

APPENDIX – C

Table C1 Single-factor ANOVA analysis for effluent COD of R1 and R2 in Phase 1

Anova: Single Factor
COD_Phase 1
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
R1	68	1487.95	21.88162	78.29072
R2	68	807.72	11.87824	79.29578

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3402.3	1	3402.3	43.1801	1.01615E-09	3.911795
Within Groups	10558.3	134	78.79325			
Total	13960.6	135				

Table C2 Single-factor ANOVA analysis for effluent COD of R1 and R2 in Phase 2

Anova: Single Factor
COD_Phase 2
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
R1	50	2699.901	53.99802	85.63783
R2	50	1361.72	27.2344	18.53847

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	17907.28	1	17907.28	343.7881	8.18546E-34	3.938111
Within Groups	5104.638	98	52.08815			
Total	23011.92	99				

Table C3 Single-factor ANOVA analysis for effluent COD of R1 and R2 in Phase 3

Anova: Single Factor
COD_Phase 3
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
R1	19	2266.86	119.3084	19.15476
R2	19	1742.13	91.69105	9.638343

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7245.831	1	7245.831	503.3033	9.44955E-23	4.113165
Within Groups	518.2758	36	14.39655			
Total	7764.107	37				

Table C4 Single-factor ANOVA analysis for effluent COD of R2 and R3 in Phase 1

Anova: Single Factor
COD_Phase 1
SUMMARY

Groups	Count	Sum	Average	Variance
R2	68	807.72	11.87824	79.29578
R3	68	1578.208	23.20894	59.1485

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	4365.086	1	4365.086	63.05911	7.18644E-13	3.911795
Within Groups	9275.767	134	69.22214			
Total	13640.85	135				

Table C5 Single-factor ANOVA analysis for effluent COD of R2 and R3 in Phase 2

Anova: Single Factor
COD_Phase 2
SUMMARY

Groups	Count	Sum	Average	Variance
R2	50	1361.72	27.2344	18.53847
R3	50	2600.72	52.0144	85.19828

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	15351.21	1	15351.21	295.9647	2.28333E-31	3.938111
Within Groups	5083.101	98	51.86837			
Total	20434.31	99				

Table C6 Single-factor ANOVA analysis for effluent COD of R2 and R3 in Phase 3

Anova: Single Factor
COD_Phase 3
SUMMARY

Groups	Count	Sum	Average	Variance
R2	19	1742.13	91.69105	9.638343
R3	19	2181.443	114.8128	14.71715

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	5078.84	1	5078.84	417.0591	2.18992E-21	4.113165
Within Groups	438.3989	36	12.17775			
Total	5517.239	37				

Table C7 Single-factor ANOVA analysis for effluent BOD_5 of R1 and R2 in Phase 1

Anova: Single Factor
BOD_5_Phase 1
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
R1	32	251.47	7.858438	14.95019
R2	32	262.45	8.201563	15.0904

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.883756	1	1.883756	0.125414	0.724437	3.995887
Within Groups	931.2584	62	15.0203			
Total	933.1422	63				

Table C8 Single-factor ANOVA analysis for effluent BOD_5 of R1 and R2 in Phase 2

Anova: Single Factor
BOD_5_Phase 2
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
R1	27	866.62	32.09704	13.97837
R2	27	794.58	29.42889	18.52501

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	96.1067	1	96.1067	5.913644	0.068504	4.026631
Within Groups	845.0878	52	16.25169			
Total	941.1945	53				

Table C9 Single-factor ANOVA analysis for effluent BOD_5 of R1 and R2 in Phase 3

Anova: Single Factor
BOD_5_Phase 3
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
R1	14	672.39	48.02786	10.28043
R2	14	586.08	41.86286	9.24736

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	266.0506	1	266.0506	27.2484	1.88212E-05	4.225201
Within Groups	253.8613	26	9.763897			
Total	519.9119	27				

Table C10 Single-factor ANOVA analysis for effluent BOD_5 of R2 and R3 in Phase 1

Anova: Single Factor
BOD_5_Phase 1
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
R2	32	262.45	8.201563	15.0904
R3	32	232.2	7.25625	12.61802

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	14.29785	1	14.29785	1.032022	0.313632	3.995887
Within Groups	858.9612	62	13.85421			
Total	873.259	63				

Table C11 Single-factor ANOVA analysis for effluent BOD_5 of R2 and R3 in Phase 2

Anova: Single Factor
BOD_5_Phase 2
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
R2	27	794.58	29.42889	18.52501
R3	27	843.9	31.25556	11.37872

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	45.0456	1	45.0456	3.012708	0.088537	4.026631
Within Groups	777.4969	52	14.95186			
Total	822.5425	53				

Table C12 Single-factor ANOVA analysis for effluent BOD_5 of R2 and R3 in Phase 3

Anova: Single Factor
BOD_5_Phase 3
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
R2	14	586.08	41.86286	9.24736
R3	14	638.7	45.62143	9.186552

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	98.88801	1	98.88801	10.72892	0.002986	4.225201
Within Groups	239.6409	26	9.216956			
Total	338.5289	27				

Table C13 Results of batch experiment study for organic removal by plants

Sample	COD (mg/L)	BOD_5 (mg/L)
Raw wastewater (T = 0 h)	205.33 ± 9.07 [9]	107.67 ± 3.79 [9]
Pot 1_control (T = 36h)	109.67 ± 5.86 [9]	66.33 ± 2.52 [9]
Pot 2_dual plant (T = 36h)	107.67 ± 1.73 [9]	66.67 ± 1.15 [9]
Pot 3_single plant (T = 36h)	109.33 ± 7.02 [9]	68.33 ± 1.15 [9]

Data format: XX ± YY [Z], where XX is mean, YY is standard deviation and Z is no. of samples tested

Table C14 Water quality criteria as per the designated best use prescribed by CPCB

Designated best use	Class of water	Criteria
Drinking WaterSource without conventional treatment but after disinfection.	Class A	• Total Coliforms Organism MPN/100ml shall be 50 or less. • pH between 6.5 and 8.5. • Dissolved Oxygen 6mg/L or more. • Biochemical Oxygen Demand 5 days 20˚C 2mg/L or less.
Outdoor bathing (Organised)	Class B	• Total Coliforms Organism MPN/100mL shall be 500 or less, pH between 6.5 and 8.5, Dissolved Oxygen 5mg/L or more. • Biochemical Oxygen Demand 5 days 20˚C 3mg/L or less
Drinking water source after conventional treatment and disinfection	Class C	• Total Coliforms Organism MPN/100mL shall be 5000 or less, pH between 6 to 9, Dissolved Oxygen 4mg/L or more. • Biochemical Oxygen Demand 5 days 20˚C 3mg/L or less.
Propagation of Wildlife and Fisheries	Class D	• pH between 6.5 to 8.5, Dissolved Oxygen 4mg/L or more. • Free Ammonia (as N) 1.2 mg/L or less.
Irrigation, Industrial Cooling, Controlled Waste disposal	Class E	• pH between 6.0 to 8.5 • Electrical Conductivity at 25˚C micromhos/cm Max.2250. • Sodium absorption Ratio Max. 26. • Boron Max. 2mg/L.
	Below Class E	• Not Meeting A, B, C, D, and E Criteria

This table is adopted from the website of CPCB (https://cpcb.nic.in/water-quality-criteria/)

micromeritics

Micromeritics Instrument Corporation

TriStar II 3020 3.02

TriStar II 3020 Version 3.02
Serial # 1623 Unit 1 Port 2

Page 1

Sample: ACTIVATED Carbon after wash
Operator: ANALYTICAL LAB CL
Submitter: IIT GUWAHATI ASSAM
File: D:\BET\Prof. BP Man...\ACTIVATED Carbon after wash.SMP

Started: 14-01-2021 11:15:03
Completed:
Report Time: 15-01-2021 09:35:43
Sample Mass: 0.4066 g
Cold Free Space: 33.7876 cm³
Low Pressure Dose: None
Automatic Degas: No

Analysis Adsorptive: N2
Analysis Bath Temp.: 77.300 K
Thermal Correction: No
Warm Free Space: 11.1343 cm³ Measured
Equilibration Interval: 10 s
Sample Density: 1.000 g/cm³

Summary Report

Surface Area

BET Surface Area: 2.4051 m²/g

Langmuir Surface Area: 3.7353 m²/g

t-Plot Micropore Area: 0.0776 m²/g

t-Plot External Surface Area: 2.3275 m²/g

BJH Adsorption cumulative surface area of pores
between 2.0000 nm and 300.0000 nm diameter: 1.173 m²/g

BJH Desorption cumulative surface area of pores
between 2.0000 nm and 300.0000 nm diameter: 1.9427 m²/g

Pore Volume

Single point adsorption total pore volume of pores
less than 195.2438 nm diameter at p/p° = 0.990000000: 0.006934 cm³/g

t-Plot micropore volume: 0.000021 cm³/g

BJH Adsorption cumulative volume of pores
between 2.0000 nm and 300.0000 nm diameter: 0.004361 cm³/g

BJH Desorption cumulative volume of pores
between 2.0000 nm and 300.0000 nm diameter: 0.002992 cm³/g

Fig. C1 Summary of BET and Langmuir surface area analysis of GAC

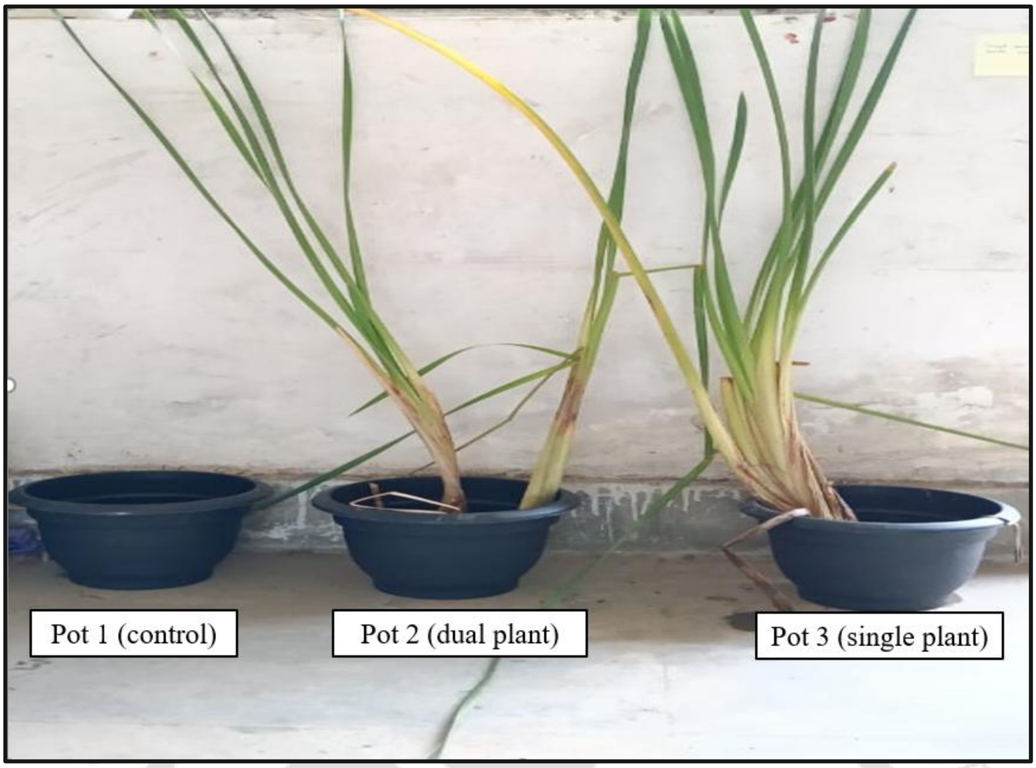

Fig. C2 Batch experiment set up for organic removal by plants with single and dual plant system

Fig. C3 Photographs of (a) & (b) Presence of root section at 10 cm depth, (c) presence of root observed at 20 cm depth, (d) & (e) roots not present at a depth of 25 cm. (f) procuring plants from R1

Fig. C4 (a) Plants obtained from reactor R1, (b) root length measurement of the plants

APPENDIX – D

Table D1 Analysis of operation and maintenance cost of the reactors for Phase 1 & Phase 2 without effluent disinfection

Labour cost (₹)		**Total cost (₹)**
Reactor assembling	Reactor maintenance	2,324
2 x 581 (for 1 day)	2 x 581 (for 1 day)	
Cost of effluent quality check		
Unit cost	No of tests p.a.	22,200
3700	6 @ interval of 50 days	
Cost of electricity for pump operation (pump rating 24 W)		
Monthly consumption (kWh)	Total consumption (kWh)	21715.2
14.98	149.76	
Total operation and maintenance cost for every reactor		**46239.2**

Table D2 Analysis of operation and maintenance cost of the reactors for Phase 1 & Phase 2 with effluent disinfection

Labour cost (₹)		**Total cost (₹)**
Reactor assembling	Reactor maintenance	2,324
2 x 581 (for 1 day)	2 x 581 (for 1 day)	
Cost of effluent quality check		
Unit cost	No of tests p.a.	22,200
3700	6 @ interval of 50 days	
Cost of electricity for pump operation (pump rating 24 W)		
Monthly consumption (kWh)	Total consumption (kWh)	21715.2
14.98	149.76	
Cost of effluent disinfection		
Chlorination (per L)	Chlorination + dechlorination (per L)	68.4
0.06	0.076	
Total operation and maintenance cost for every reactor		**46307.6**

Table D3 Cost of unit power generation for the reactors in Phase 1 and Phase 2

Without effluent disinfection	**Reactors**	**Operation and maintenance cost**	**Power density (W/m^2)**	**Cost per unit power generation ₹/((W/m2)/d)**
Phase 1	R1	46239.2	N.A.	N.A.
	R2	46239.2	0.00465	717.30
	R3	46239.2	0.00132	2138.02
Phase 2	R1	46239.2	N.A.	N.A.
	R2	46239.2	0.00296	981.06
	R3	46239.2	0.00097	2677.53
With effluent disinfection	**Reactors**	**Operation and maintenance cost**	**Power density (W/m^2)**	**Cost per unit power generation ₹/((W/m2)/d)**
Phase 1	R1	46239.2	N.A.	N.A.
	R2	46239.2	0.00465	717.53
	R3	46239.2	0.00132	2138.25
Phase 2	R1	46239.2	N.A.	N.A.
	R2	46239.2	0.00296	981.28
	R3	46239.2	0.00097	2677.76

N.A. not applicable

Table D4 Benefit model analysis for the reactors without effluent disinfection

Phase 1	Reactors	Electrode area (m^2)	Power density (W/m^2)	R_{pe} (₹)
Revenue from bioelectricity generation (R_{pe})	R1	N.A.	N.A.	0.00
	R2	0.0196	0.00465	0.38
	R3	0.0196	0.00132	0.11
Revenue from wastewater treatment (R_{tw})	**Reactors**	**Reactor volume (mL)**	**Revenue / m^3**	**R_{tw} (₹)**
	R1	4500	0.00	0.00
	R2	4500	5.19	4.67
	R3	4500	0.00	0.00
Revenue per unit power production (R_{pu})	**Reactors**	**R_{pe}**	**R_{tw}**	**R_{pu} (₹)**
	R1	0.00	0.00	0.00
	R2	0.38	4.67	1086.24
	R3	0.11	0.00	81.82
Phase 2	**Reactors**	**Electrode area (m^2)**	**Power density (W/m^2)**	**R_{pe} (₹)**
Revenue from bioelectricity generation (R_{pe})	R1	N.A.	N.A.	0.00
	R2	0.0196	0.0042	0.34
	R3	0.0196	0.0011	0.09
Revenue from wastewater treatment (R_{tw})	**Reactors**	**Reactor volume (mL)**	**Revenue / m^3**	**R_{tw} (₹)**
	R1	3000	5.19	0.00
	R2	3000	5.19	0.00
	R3	3000	5.19	0.00
Revenue per unit power production (R_{pu})	**Reactors**	**R_{pe}**	**R_{tw}**	**R_{pu} (₹)**
	R1	0.00	0.00	0.00
	R2	0.34	0.00	81.93
	R3	0.09	0.00	84.11

N.A. not applicable

Table D5 Benefit model analysis for the reactors with effluent disinfection

Phase 1	**Reactors**	**Electrode area (m^2)**	**Power density (W/m^2)**	**R_{pe} (₹)**
Revenue from bioelectricity generation (R_{pe})	R1	N.A.	N.A.	0.00
	R2	0.0196	0.00465	0.38
	R3	0.0196	0.00132	0.11
Revenue from wastewater treatment (R_{tw})	**Reactors**	**Reactor volume (mL)**	**Revenue / m^3**	**R_{tw} (₹)**
	R1	4500	5.19	4.67
	R2	4500	5.19	4.67
	R3	4500	5.19	4.67
Revenue per unit power production (R_{pu})	**Reactors**	**R_{pe}**	**R_{tw}**	**R_{pu} (₹)**
	R1	N.A.	4.67	N.A.
	R2	0.38	4.67	1086.24
	R3	0.11	4.67	3620.45
Phase 2	**Reactors**	**Electrode area (m^2)**	**Power density (W/m^2)**	**R_{pe} (₹)**
Revenue from bioelectricity generation (R_{pe})	R1	N.A.	N.A.	0.00
	R2	0.0196	0.0042	0.34
	R3	0.0196	0.0011	0.09
Revenue from wastewater treatment (R_{tw})	**Reactors**	**Reactor volume (mL)**	**Revenue / m^3**	**R_{tw} (₹)**
	R1	3000	5.19	4.67
	R2	3000	5.19	4.67
	R3	3000	5.19	4.67
Revenue per unit power production (R_{pu})	**Reactors**	**R_{pe}**	**R_{tw}**	**R_{pu} (₹)**
	R1	N.A.	N.A.	0.00
	R2	0.34	0.00	1207.27
	R3	0.09	0.00	4449.53

N.A. not applicable

F.No.1/4(3)/2022-LS-II
Government of India
Ministry of Labour& Employment
Office of the Chief Labour Commissioner(C)
New Delhi

Dated:29/07/2022

ORDER

In supersession to this office order of even reference dated 31st March 2022 and in exercise of the powers conferred by Central Government vide Notification No. S.O. 188(E) dated **19th January, 2017** of the Ministry of Labour and Employment the undersigned hereby revise the rates of Variable Dearness Allowance on the basis of the average Consumer Price Index for Industrial workers reaching 357.65 from 345.22 as on 31.12.2021 (Base 2016-100) and thereby resulting in an increase of 12.43 points. The revised Variable Dearness Allowance as under shall be payable from 01.4.2022;-

The rates of Variable Dearness Allowance for employees employed in **CONSTRUCTION OR MAINTENANCE OF ROADS OR RUNWAYS OR IN BUILDING OPERATIONS INCLUDING LAYING DOWN UNDERGROUND ELECTRIC, WIRELESS, RADIO, TELEVISION, TELEPHONE,TELEGRAPH AND OVERSEAS COMMUNICATION CABLES AND SIMILAR OTHER UNDERGROUND CABLING WORK, ELECTRIC LINES, WATER SUPPLY LINES AND SEWERAGE PIPE LINES.**

Category of worker	Rates of V.D.A. Area wise per day (in Rupees)		
	A	B	C
Unskilled	172	148	116
Semi-Skilled/Unskilled Supervisory	191	162	134
Skilled/Clerical	209	191	164
Highly Skilled	224	209	191

Therefore the minimum rates of wages showing the basic rates and Variable Dearness Allowance payable w.e.f. 01.4.2022 will be as under :-

Category of worker	Rates of wages including V.D.A. per day (in Rupees)		
	A Area	B Area	C Area
Unskilled	523+172=695	437+148=581	350+116=466
Semi-Skilled/Unskilled Supervisory	579+191=770	494+162=656	410+134=544
Skilled/ Clerical	637+209=846	579+191=770	494+164=656
Highly Skilled	693+226=919	637+209=846	579+191=770

The VDA has been rounded off to the next higher rupee as per the decision of the Minimum Wages Advisory Board.

The classification of workers under different categories will be same as in Part-I of the notification, whereas classification of cities will be same as in the Part-II of the notification dated 19th January, 2017. The present classification of cities into areas A, B & C is enclosed at Annexure I for ready reference.

29/7/22
(S.C.Joshi)
Chief Labour Commissioner(C)

Fig. D1 Minimum rate of wages for the labours as recommended by the Ministry of Labour and Employment in India.

D1. Rates and tariffs associated with CBA analysis in the current study

1. Cost of land in Guwahati (The location, where the reactors were installed) = ₹ 3242 / ft^2

2. Daily labour cost = ₹ 581 per person per day (for unskilled labours)

3. Tariff of electricity charge = ₹ 145 / kWh / month (as per Assam state electricity board tariff, Guwahati, Assam, India)

4. Price of irrigation water in India for rice = ₹ 5.19/m^3

5. Cost of chlorination below 8 MLD = ₹ 0.06/L

6. Cost of chlorination + dechlorination below 8 MLD = ₹ 0.076/L

Table D7 Quality assessment of raw wastewater and treated effluent during the study without effluent disinfection

Reactors and Phases		**Pre and post-treatment wastewater characteristics**				
		COD (mg/L)	DO (mg/L)	pH	FC (log) (MPN/100 mL)	SAR
Phase 1	Feed	166.2±9.4	N.D.	7.1±0.4	8.5±0.3	0.95±0.11
	R1	20.8±8.5	2.2±0.2	7.6±0.1	3.8±0.4	0.62±0.06
	R2	13.9±3.5	1.9±0.1	7.5±0.1	2.7±0.3	0.87±0.12
	R3	22.2±7.1	0.7±0.1	7.7±0.2	3.6±0.3	0.77±0.14
Phase 2	Feed	202.4±8.3	N.D.	7.2±0.2	8.7±0.6	0.81±0.17
	R1	59.5±3.5	2.0±0.1	7.3±0.	3.8±0.3	0.69±0.09
	R2	26.2±2.5	1.7±0.2	7.6±0.2	3.3±0.1	0.83±0.16
	R3	57.3±3.1	0.4±0.1	7.5±0.1	3.5±0.2	0.78±0.16

The format xx±yy; where x is the mean, and y is the standard deviation. FC: Fecal coliforms, SAR: Sodium adsorption ratio, N.D.: Not detected.

APPENDIX – E

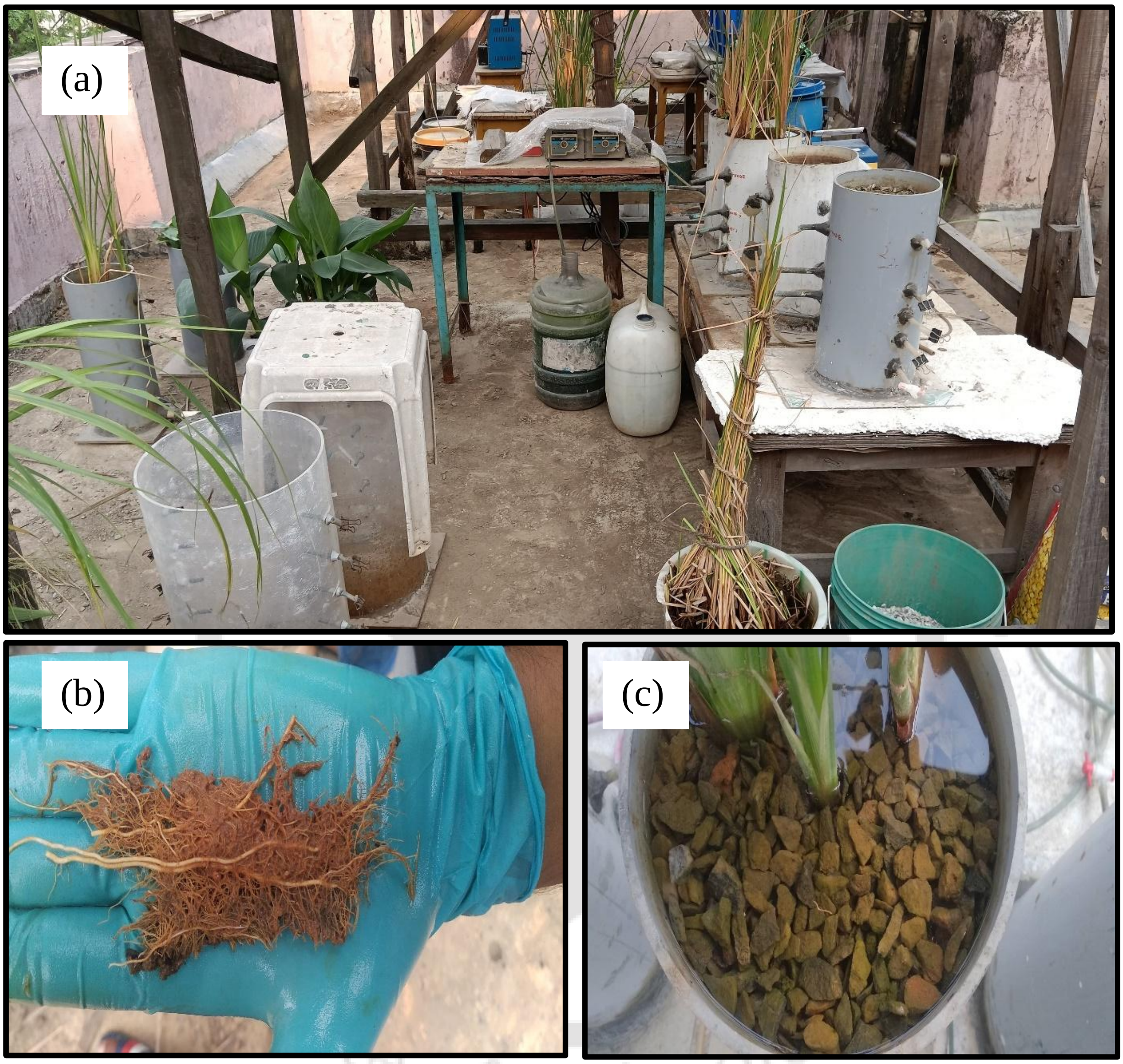

Fig. E1 Photographs of (a) Reactor setup, (b) Root section obtained from reactor R2, and (c) Effluent of reactor R2

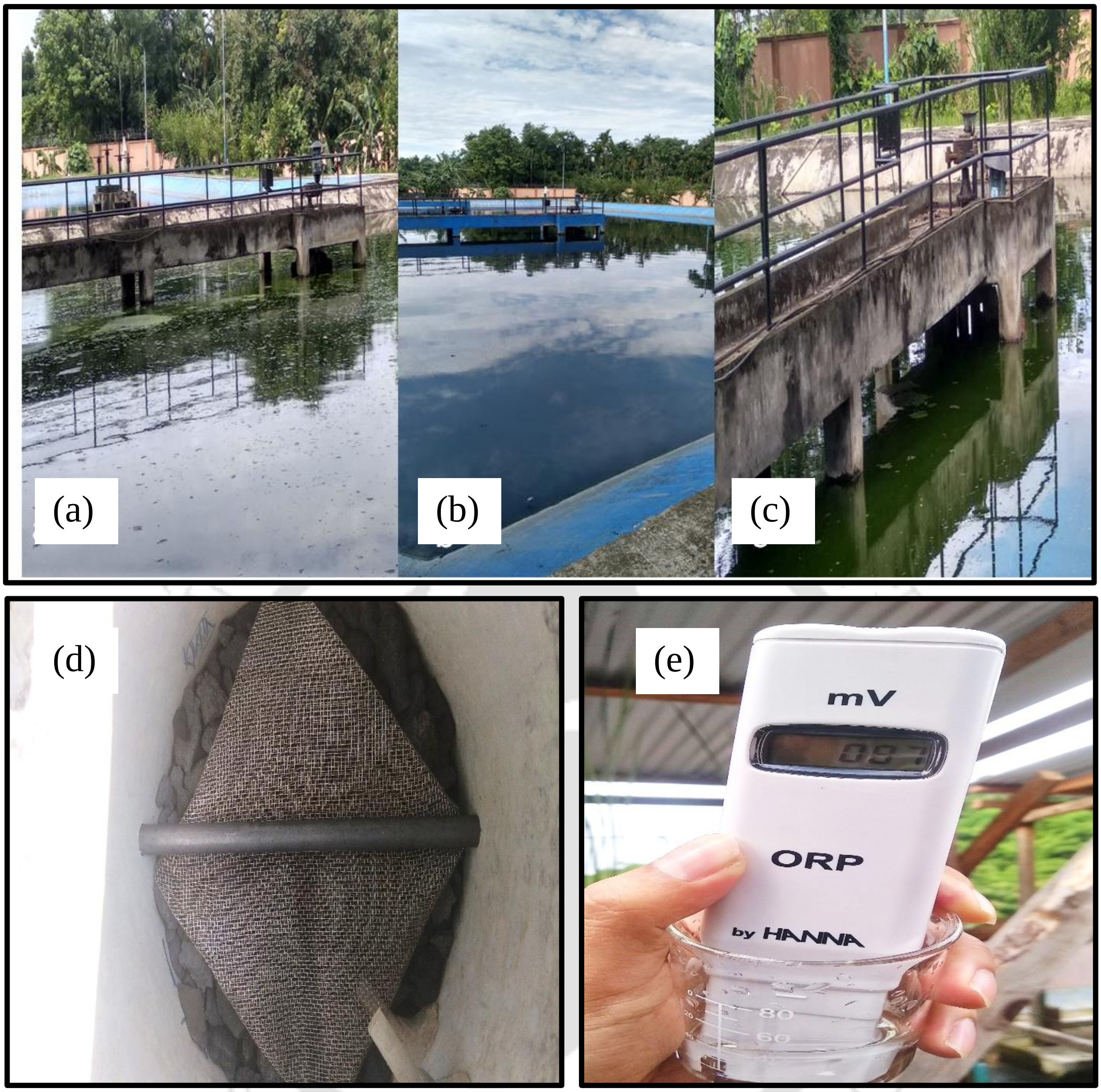

Fig. E2 Photographs of (a, b, c) Sewage treatment plant in IIT Guwahati, (d) Stainsless steel mesh and graphite rod as the anode in reactor R2 during fabrication, and (e) Measurement of effluent ORP.

Table E1 List of the major instruments used in the research

Instrument	Model/manufacturer and specification
COD digester	HACH, USA
Digital pH meter	Systronics 361, India
Electronic digital balance	Citizen CX 220, India
Field emission scanning electron microscope (FESEM)	Gemini 300, Zeiss, Germany
Flame photometer	Systronics 128, India
Horizontal Shaker	Reico, India
Hot air oven	ICT, India
Incubator	HTLP (HTLP-020), India
Laboratory autoclave	Reico (RA), Anjishnu
Muffle Furnace	Multispan, India
Peristaltic pumps	BTS 50, India
Portable digital ORP meter	HANNA, India
Refrigerator	Labocon, LMR-102, India
Visible spectrophotometer	Systronics, India
Water purification system	Merck, Germany
X-ray powdered diffraction	Rigaku smart lab X-ray diffractomter
Weather station	Davis Vantage Pro, USA

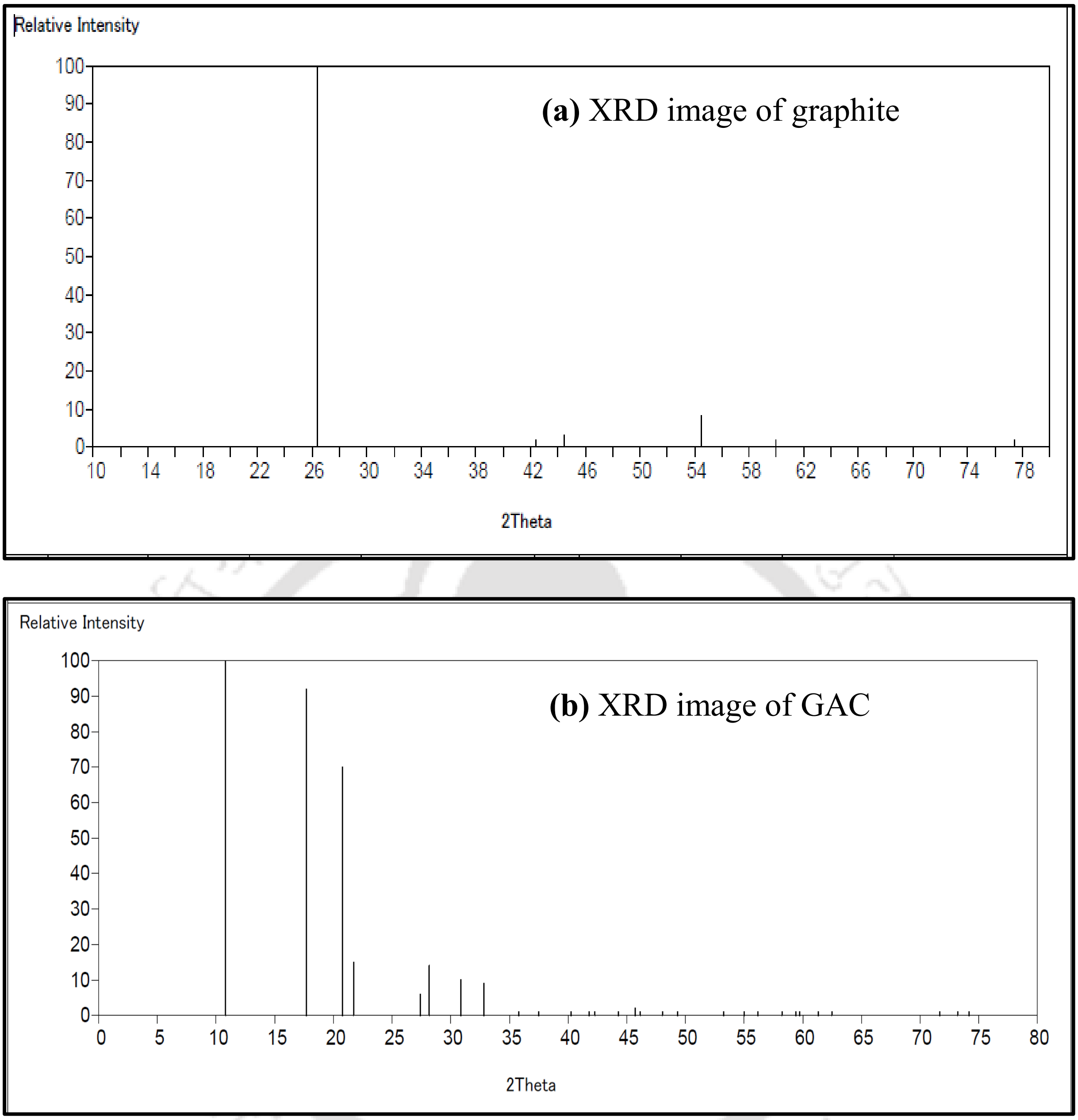

Fig. E3 XRD images of (a) graphite and (b) GAC used in the current resarch

Publications and Awards

Research articles

1. **Biswas, A.**, & Chakraborty, S., 2023. Assessment of microbial population in integrated CW-MFC system and investigation of organics and fecal coliform removal pathway. *Science of The Total Environment, 60*, 103475. doi.org/10.1016/j.jclepro.2023.137204.
2. **Biswas, A.**, & Chakraborty, S., 2023. Cost benefit analysis of integrated constructed wetland microbial fuel cell system for sustainable and economic domestic wastewater treatment. *Sustainable Energy Technologies and Assessment*, *60*, 103475. doi.org/10.1016/j.seta.2023.103475.
3. **Biswas, A.**, & Chakraborty, S., 2023. Organics and coliform removal from low strength domestic wastewater using integrated constructed wetland - microbial fuel cell reactor with bioelectricity production. *Journal of Cleaner Production*, *408*, 137204. doi.org/10.1016/j.scitotenv.2023.168809.
4. **Biswas, A.** & Chakraborty, S., 2025. Variation in bioelectricity production in integrated CW-MFC: An insight into coliform inactivation affected by HRT and power density. *Journal of Environmental Management*, *373*, p.123527. doi.org/10.1016/j.jenvman.2024.123527.

Manuscript under review/under preparation

1. **Biswas, A.**, & Chakraborty, S. (2024). Power density enhancement in multi-anode CW-MFC and inactivation of E coli under different circuit operations. **(Under preparation)**

Book chapter

1. **Biswas, A.**, & Chakraborty, S. (2021). Environmental remediation using integrated microbial electrochemical wetlands: iMETLands. *In Applied Water Science: Remediation Technologies, Volume 2* (pp.171-190). Wiley online library. doi.org/10.1002/9781119725282.ch4.

Conferences and workshops attended

1. 3rd International Conference on Water Technologies (ICWT-2023) AUA Academy. **Biswas, A.**, & Chakraborty. S. *Coliform Removal in Low Strength Domestic Wastewater using Microbial Fuel Cell: A Seasonal Analysis* Indian Institute of Technology Bombay. December 2023.
2. Research and Industrial Conclave (RIC-2023), Guwahati, Assam, India. **Biswas, A**., & Chakraborty, S. Assessment of sewage treatment plant of IIT Guwahati: A case study. Indian Institute of Technology, Guwahati. May 2023.
3. 9th International Conference on Sustainable Waste Management towards Circular Economy, (9th IconSWM-CE-2019), Bhubaneswar, India. **Biswas, A**., Chakraborty, S. Evolution and recent research trends in constructed wetland mediated environmental remediation and future prospects: Review Organised by ISWMAW, KIIT, Bhubaneswar, November 2021.
4. Five days national workshop on *Technological Emergence on Clean Water and Air* **(TECWA-2023),** National Institute of Technology Rourkela

Awards and accolades from current research

1. **Best Presentation Award** by **Springer Nature** in the category of Processes (Biochemical and Bioremediation). Water Technologies (ICWT 2023), Indian Institute of Technology Bombay.
2. **Best Presentation award** under Department of Civil Engineering: Research and Industrial Conclave (RIC-2023), Indian Institute of Technology Guwahati.
3. **AWSAR** award for popular science stories under Ph.D. Category in 2022. Awarded by **DST India**.