Integrated Hydroponics-Microbial Electrochemical Technology for Simultaneous Wastewater Treatment, Plant Cultivation, and Energy Recovery

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of BS-MS dual degree in Science

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Certificate of Examination

This is to certify that the dissertation titled "Integrated Hydroponics-Microbial Electrochemical Technology System for simultaneous Wastewater Treatment, Plant Cultivation, and Energy Recovery" submitted by Mr. Ravi Kumar Yadav (Reg. No. MS14090) for the partial fulfilment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

Dr. Santosh B. Satbhai	Dr. Anoop Ambili	Dr. Sunil A. Patil (Supervisor)

Dated: April 22,2019

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr. Sunil A. Patil at the Indian Institute of Science Education and Research Mohali.

This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Ravi Kumar Yadav

Dated: April 22, 2019

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Sunil A. Patil
(Supervisor)

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Ravi Kumar Yadav

List of Figures

List of Photographs

List of Tables

Abbreviations

MLD- Million Liters per Day
BOD- Biological Oxygen Demand
MFC- Microbial Fuel Cell
CWs- Constructed Wetlands
PVC- Polyvinyl Chloride
CPVC- Chlorinated Polyvinyl Chloride
WC- Without Cocopeat
C- Cocopeat
C+E- Cocopeat with Electrode
I-O- Integrated Open circuit system
I-P- Integrated Parallel connected system
I-S- Integrated Parallel connected system
DS- Domestic Sewage
N and P- Nitrogen and Phosphorus
HRT- Hydraulic Retention Time
OCV- Open Circuit Voltage
COD- Chemical Oxygen Demand
MPN- Most Probable Number
S1- System1
S2- System2
PAOs- Phosphate Accumulating Organisms
STP- Sewage Treatment Plants

Contents

Bibliography

Abstract

Rapid urbanization has led to an increase in the production of domestic wastewater thereby putting enormous pressure on the existing centralized sewage treatment plants. Upgrading the existing treatment plants is not easy due to various technical challenges and high costs. Decentralized wastewater treatment systems are therefore emerging as one of the alternative strategies for wastewater treatment at the point sources. Most of the available technologies commonly employ separate treatment processes for the removal of organic matter and nutrients from the wastewaters. Moreover, they are also not equipped with resource recovery capabilities. The available wastewater treatment processes are either energy or chemical intensive, costly, and suffer due to operational complexity. The development of decentralized integrated systems based on multiple processes is anticipated/hypothesized to address some of these issues. In this context, we tested a decentralized wastewater treatment system based on the integration of drip hydroponics and microbial electrochemical technologies not only for wastewater treatment but also for resource recovery in the form of plant cultivation and energy production. In a proof-of-principle design, the integrated system consisted of ten reactor units, and each unit housed a graphite anode embedded in the cocopeat support media bed, a graphite air cathode, and *Cymbopogon citratus* grass (common name: lemongrass). The system was operated in a semi-continuous drip-hydroponics mode. Various wastewater treatment parameters, which include COD, BOD, ammonia, nitrate, phosphate, coliforms, pH, and conductivity along with the electricity output in terms of cell voltage were monitored continuously for evaluating the performance of the system. At 3 h hydraulic retention time (HRT), the integrated hydroponics-microbial electrochemical system achieved 72% COD, 80% phosphates and 35% ammonia removal efficiencies. In addition to the wastewater treatment, the presented system produced low levels of electricity output and allowed simultaneous cultivation of commercially important plants. Up to 30.9 mW/cm^2 and 31.6 mW/m^2 power outputs were obtained with a serially and parallelly connected microbial fuel cell units in these systems, respectively. At longer HRTs of 6 h or 12 h, more than 85% COD and N & P nutrients removal efficiencies were observed. As

hypothesized the innovative integration in a single system allowed the exploitation of activities of multiple biological components such as aerobes, anaerobes, exoelectrogens, plant roots and rhizosphere microbiota for the removal of different pollutants present in domestic wastewater. The proof-of-principle study demonstrated the feasibility of the integrated system for efficient domestic wastewater treatment with simultaneous electricity generation and commercially important plant cultivation. Other unique features of the proposed system include easy operation, no chemicals, no foul odor, and CO_2 sequestration by plants.

Chapter 1

Introduction

1.1 Current status of domestic wastewater generation and treatment

Water is an essential resource for the existence of all life forms including humans. All the industrial and irrigational processes implemented to produce a spectrum of products from fuel to food are water dependent. Due to the increase in human population and extensive use of water resources, water is increasingly becoming one of the scarcest resources. It is estimated that nearly two-thirds of the global population (i.e., 4 billion people) will live under conditions of severe water scarcity at least for a month in a year (Mekonnen et al., 2016). Non-conventional sources such as wastewaters are thus now seen as the potential resources for obtaining usable water. Another issue with the rising population is the rapid urbanization, which is leading to increased domestic wastewater production thereby putting enormous pressure on the existing centralized sewage treatment plants. Development of wastewater treatment infrastructures in India occurred at slow pace, thereby leaving a significant fraction of the population without adequate access to water and sanitation services. Globally, the average treatment of municipal and industrial wastewater is around 70 % in high-income, 38% in upper-middle-income, 28 % in lower-middle-income, and 8 % in low-income countries (ONU, 2017). It is clear that more than 80% of wastewater is discharged into the environment without negligible or any treatment globally. If we consider only class-II towns within India, only about 34.74% of 75,020 MLD (million liters per day; data for 2018) sewage generated can be treated with existing wastewater treatment facilities and available technical support (www.indianinfrastructure.com) (Fig. 1). Apart from the domestic wastewater, a considerable volume of agricultural runoff and other wastewaters also pollute freshwater reservoirs on a massive scale, resulting in significantly less safe freshwater for human use in urban as well as rural settlements.

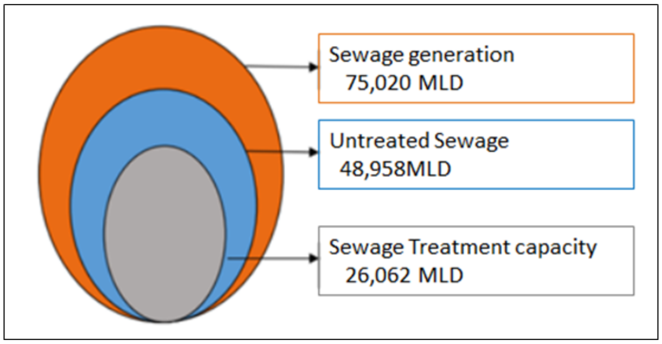


Figure 1. National status of sewage generation and treatment.

1.2 Existing wastewater treatment technologies: pros and cons

There are many conventional wastewater treatment technologies based on biological processes such as anaerobic digestion, aerobic treatment/digestion, and nutrient removal approaches. Aerobic treatment is fast and efficient but produces high sludge and is energy intensive because of continuous aeration required for aerobic microbes to degrade organic matter present in the wastewater (Sandra, et al., 2017). Anaerobic digestion can treat the wastewater and simultaneously produce bioenergy in the form of biogas, but these systems are slow because of the slow growth rate of anaerobic microbes and generally requires high Biological Oxygen Demand (BOD) strength influent for efficient and economical treatment (Iit and Web, 2011). The nutrient (N & P) removal processes commonly referred to as biological nutrient removal processes are based on the combination of different growth conditions (such as aerobic and anoxic) and microbial groups in multiple process units, and are thus not cost-effective (Breidt, 2015). Microbial electrochemical technologies such as microbial fuel cell (MFC) systems are emerging as novel wastewater treatment approaches as they can be used to treat a wide range of pollutants (organic and inorganic) through bioelectrochemical reactions and energy in terms of electricity can be produced (Pandey et al. 2016). Those microorganisms which are capable of extracellular electron transfer, commonly referred to as electroactive microorganism are critical to the functioning and performance of MFC systems (Vatsysyan, 2014). Their implementation for large scale wastewater treatment applications is, however, not economical yet because of high material or reactor costs and low power outputs (Chen et al. 2018).

Other technologies such as constructed wetlands (CWs), which have been offering innovative solutions for wastewater treatment and water management for several decades, are based on phytoremediation principles. Integration of microbial fuel cell principles in CWs has been a recent major intervention in CWs technology (Yadav et al. 2018); (Wei et al. 2015). These systems can be used for decentralized wastewater treatment as they offer significant advantages over other conventional wastewater treatment methods. Even though CWs technology is well-established, it is not widely applied to treat various wastewaters mainly because it requires a large land area and suffers due to clogging issues, (Doherty et al. 2015).

Overall, the conventional wastewater treatment processes are either energy or chemical intensive, costly, and suffer due to operational complexity. Moreover, most of the existing technologies do not offer any opportunities for the recovery of various resources present in wastewaters.

1.3 Strategies and innovative approaches to treating domestic sewage

Considering the various issues associated with the conventional technologies and upgradation challenges of the existing treatment plants, there is an urgent need for the development and implementation of real low-cost but efficient technologies at large scales and in large numbers. Exploration of the decentralized approach is one of the primary essential strategies to solve the various problems of the existing centralized treatment plants (Cai et al. 2012). Adapting more than one low cost, easily implementable and manageable treatment technologies in an integrated manner is another strategy for wastewater treatment and resource recovery.

In this context, several researchers are working on developing integrated technologies based on various processes. The integration of microbial fuel cells in CWs and anaerobic digestion technologies is a recent development (Borràs et al. 2015); (Srivastava et al. 2018). Recently, ecological engineering systems have also emerged as a decentralized and sustainable wastewater treatment approach. The designed ecological engineered system mimics the natural cleansing functions of wetlands for wastewater treatment. A typical ecological engineering system consists of three different tanks interconnected in a series. The wastewater flows by gravity. The system houses diverse biota, viz., aquatic macrophytes, submerged plants, emergent plants and filter feeders for wastewater treatment through ecological interactions and self-purification (Chiranjeevi et al., 2013); (Mohan et al. 2009). These systems have been reported to be ecologically complex but are mechanically simple and have very low energy consumption. However, the fulfilment of the paramount need of recovering, recycling, and reusing the resources such as water, nutrients, and energy from wastewaters in a single operational unit sustainably and ecologically with quick adaptability is still a main challenge with such technologies.

1.4 Hydroponics technologies for plant cultivation

Soil is not a requisite thing for growth of all plants. The plant growth and development depend immensely on the availability and uptake of essential macro and micro nutrients. Soil act as a media for nutrient availability in addition to providing mechanical support to plants. The hydroponic technique is a subset of hydroculture, in which plants are grown without soil support in a mineral nutrient-rich water solution. The plant roots are directly exposed to the mineral solution, or supported by the inert media such as cocopeat, perlite, gravels, vermiculite, rockwool, etc. (Pradhan and Deo, 2019). So, several hydroponics based agricultural techniques (e.g., aquaponics and aeroponics) have been developed to address the issues of infertility of soil, lack of arable land, water scarcity, climatic hurdles in agriculture, and leaching of fertilizers in soil. However, they also face some limitations such as high capital cost, continuous monitoring, and requiremnt of expensive nutrient solution and water (Pandey et al., 2009). To address some of these issues, a few studies have demonstrated the use of domestic sewage as a the nutrient medium (K. A., 2018).

1.5 Proposed solution

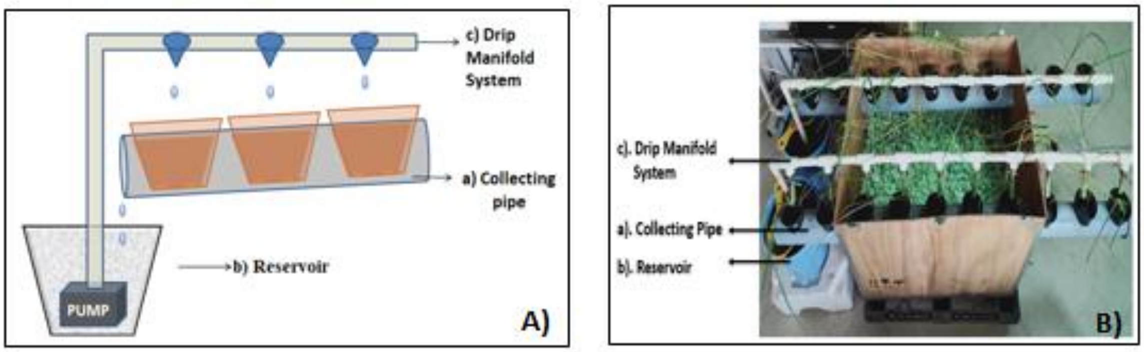
To address the limitations of the existing wastewater treatment as well as hydroponics technologies as discussed above, we propose an integrated Hydroponics-Microbial Electrochemical Technology for decentralized wastewater treatment, and simultaneous energy recovery and plant cultivation. It is based on the use of multiple reactor units, each unit consisting of the support carrier bed material, electrodes, and plants. In principle, it utilizes the processes of the aerobic, anoxic, microbial fuel cell, and hydroponics technologies in one reactor unit (Fig. 2). We hypothesize that the unique arrangement and operation of the integrated system allow the proliferation of different microbiota with diverse functionalities in different microenvironments (e.g., aerobic, anaerobic and rhizosphere) and thereby contribute to the removal of different pollutants. For instance, aerobes and anaerobes can act upon the organic matter present in wastewater. Electroactive microorganisms growing at the anode oxidize organic carbon (present in wastewater and root exudates) and release electrons that can be harvested in the form of electricity. Whereas, nutrients such as N and P can be taken up by the plants via root systems, and also converted by different groups of bacteria in different microenvironments. The main aim of this thesis was to develop and test an integrated Hydroponics-Microbial Electrochemical Technology System for decentralized wastewater treatment with simultaneous plant cultivation and energy recovery.

Chapter 2

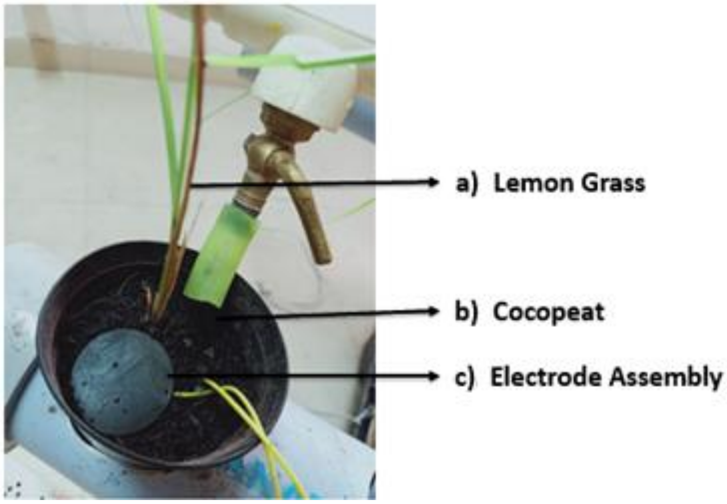
Materials and Methods

2.1 Configuration of the Integrated system

Two identical systems were designed and custom-made using different materials. Each system consisted of three major components (Photograph 1). These include: a) polyvinyl chloride (PVC) pipe (3” dia) as the main structure for the placement of 10 reactor units/pots and effluent collection; b) Plastic container (10 L capacity) used as a reservoir with a submersible pump (15 W capacity, maximum 650 L/h flow rate) used for pumping and recirculating wastewater; and c) Drip manifold made up of chlorinated polyvinyl chloride (CPVC) pipe (2 cm dia) equipped with an adjustable drip valves (for each reactor unit) at equal distance for dripping of wastewater . One end of the manifold was connected to the submerged pump placed in the reservoir to pump wastewater to each pot/reactor unit through the manifold drip system. The effluent from the reactor units was allowed to drain through small holes into the main PVC pipe and was collected in the reservoir tank. Each pot or reactor unit was cylindrical shaped and made up of plastic (7.5 cm dia) with a total/working volume of 250/200 ml.



Photograph 1. Schematic (a) and original pictures showing three basic components of the proposed system.



Photograph 2. Reactor unit configuration

Each reactor unit of the integrated system housed the plant sapling, cocopeat carrier/support bed material, and two electrodes (anode and cathode) (Photograph 2). The selection of these reactor materials are described in the following sections.

2.2 Plant selection

Cymbopogon citratus, which belongs to the *Gramineae* family and worldwide known as lemongrass, was used for the plant cultivation studies in the integrated system. Its characteristic lemon-like odor owes the 'lemon' prefix, which is mainly due to the presence of a cyclic monoterpene named "citral" (Qadir and Fatima, 2018). It is a fast-growing, tufted perennial aromatic grass which can grow up to a height of at least 1 meter with numerous stems with stiff leaves arising from short rhizomatous roots. It bears linear, green leaf-blades which are tapered at both ends. It is a monocot C_4 plant having kranz leaf blade anatomy and low CO_2 compensation point (Bertea et al. 2003). Though adapted very well to temperate climates, *C. citratus* can grow in varying environmental conditions. It is an economically important plant with a reasonable lifespan of around five years. It is used extensively in Ayurvedic medicines. It has been suggested to possess various pharmacological activities such as anti-amoebic, anti-bacterial, anti-diarrheal, anti-filarial, anti-fungal and anti-inflammatory properties (Qadir and Fatima, 2018). Its biomass is used to produce commercially valuable essential oils and in food technology as well as in traditional medicine. The above-listed features of this plant favoured its selection and use in our experiments. The saplings of lemongrass were obtained from a nearby nursery (Shri Ajit Nursery, Phase-9, Mohali). Before the transplantation into the reactor units, their roots were washed 2-3 times by using tap water and cut into identical size in terms of dimension (Total length: 57cm, Root system length: approx. 10cm) (Photograph 3). Each sapling was placed near the anode in each reactor without disturbing their root-system.

Photograph 3. Lemon grass plants with similar dimensions.

2.3 Carrier material

We used cocopeat as the carrier material in the reactor units. Cocopeat is an organic, fibrous agricultural by-product obtained from the coconut husk. It is a renewable and environment friendly media. Recently, coconut coir has been increasingly used in horticulture as a cultivation substrate due to its stable physicochemical and biological properties (Xiong et al. 2017). It can resist a wide range of pH, electrical conductivity and other chemical attributes, which make its use as soilless carrier material advantageous in hydroponic systems (Awang et al. 2009). It has high porosity and water holding capacity which limits the air-water relationship, and finally lowers the aeration within the medium, thus prohibits the oxygen diffusion to the roots and make the environment completely anaerobic (Awang et al. 2009). The high cation exchange capacity of the material allows the nutrient absorption on its surface and release to the plant when required. It also supports the growth of native bacteria and fungi (Canada et al. 2017). Hence, it acts as a perfect growth media and provides sufficient anchorage to the plants. It was also obtained from the nearby nursery (Shri. Ajit Nursery, Phase-9, Mohali). Before use, it was washed three times with tap water to remove the impurities and dust followed by drying at 70 ^{0}C, and sieved to get uniform particle size (2–3 mm). For uniformity, 12 g of dried cocopeat was used as the support bed or carrier materials in each reactor unit (Photograph 4).

Photograph 4. Reactor unit/Pot filled with 12g of cocopeat.

2.4 Electrode material

Carbon-based materials possess unique properties such as good electrical conductivity, non-corrosive, chemical resists, biocompatibility, cheap, easy availability, and non-corrosive (Corb et al. 2007), which make them most suitable and preferred electrode materials for microbial electrochemical studies (Kalathil et al. 2017); (Guo et al. 2015). Non-catalysed disc-shaped graphite electrodes with similar dimensions (40 mm diameter and 5 mm thickness; projected surface area of 12.56 cm^2 (Photograph 5)) were used as both anode and cathode in the reactor units. Before use, all electrodes were polished with sand paper, pre-treated with 1N hydrochloric acid for 2 h to remove the impurities followed by soaking in distilled water for 1 h and drying at 60º C in hot air oven (Feng et al. 2010). Copper wires of 1 mm thickness were used as the current collectors and were connected with the electrodes using conductive cement. The resistance of each electrode was 2±1 ohms. An electrode placed within the cocopeat horizontally acted as an anode and electrode placed on the surface of the cocopeat served as an open-air cathode in each reactor unit. Thus, each reactor unit acted as a single microbial fuel cell unit. To minimize the electric resistance,, the anode to cathode distance was maintained at around 1 cm in each reactor unit.

Photograph 5. Disc-shaped graphite electrodes.

2.5 Experiments conducted to evaluate the performance of the integrated system

The integrated hydroponic microbial electrochemical technology system was evaluated for domestic wastewater treatment under different reactor conditions, viz., without cocopeat (WC), with cocopeat (C), cocopeat and electrode assembly (C+E) and finally, the integrated design comprised of cocopeat, electrodes, and plants (I). Two systems (S1 and S2) were operated under a similar set of experimental conditions at ambient temperature (22 ± 2 °C), and in continuous batch-recirculation drip-hydroponics mode. Daily 20 L of fresh domestic sewage (DS) was collected at around 10 am from the sedimentation tank (primary treatment unit) of the sewage treatment plant facility located at IISER Mohali residential campus. In each experimental run, 10 L DS was fed to the system, which dripped to each reactor unit at a flow rate of 8 L/h. The performance at each reactor condition was evaluated by operating both systems at a hydraulic retention time (HRT) of 3 h. Both systems were fed with fresh wastewater at least for three/four days and the effluent samples were analyzed at least for three/four times at different set of reactor conditions (WC, C, C+E and I). Before the analyses at each condition, the systems were stabilized for a week or two weeks as required. The performance of the integrated systems was also evaluated at longer HRTs of 6 and 12 h for the removal of various pollutants and electricity generation in terms of Open Circuit Voltage (OCV) and power outputs. The plant biomass growth was measured by weighing the leaf dry mass.

2.6 Analyses

2.6.1 Wastewater parameters

The wastewater treatment performance of the integrated system was assessed by monitoring pH, conductivity (Oakton PC2700 pH/ Conductivity meter), Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD_5) (Oxitop BOD, incubation at 20 ^{0}C), ammonia, nitrate, phosphate, and coliforms (by Most probable number - MPN test) using standard protocols adapted from "*Standard Methods for the Examination of Water and Wastewater"* American Public Health Association (APHA, 2012). The treatment performance was evaluated by estimating removal efficiencies (ξ) calculated using Eq. (2), where, C_O represents the initial concentration of the specific pollutant in the infleunt, and C_S denotes concentration of the same pollutant (mg/l) in the effluent.

$$\xi = \frac{C_O - C_S}{C_O} \times 100 \qquad \text{------------ (2)}$$

Standard calibration curves for COD, nitrate, ammonia, and phosphate at different concentrations were plotted using spectrophotometry (Photolab 7600 UV-VIS). The standard curves for these parameters are presented below (Fig 2).

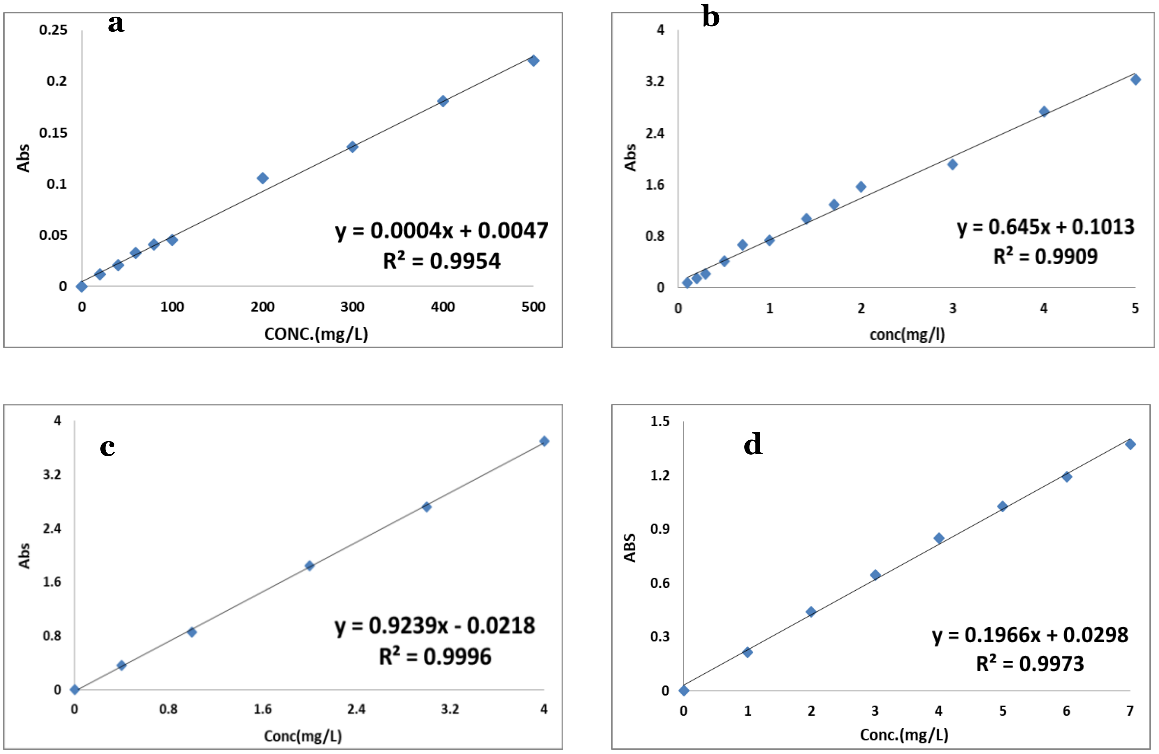


Figure 2. Standard curves for a) COD at 600nm, b) phosphate at 690nm, c) ammonia at 640nm, and d) nitrate at (A220 - A275nm)

The data is presented as an average along with uncertainties based on at least three analyses for each parameter in case of all experiments.

2.6.2 Analyses of the electrochemical parameters of the microbial fuel cells

The MFC units in the C+E as well as complete integrated (I) systems were operated initially in an open circuit mode (I-O). Open circuit voltage (OCV) was recorded using the auto-range digital multimeter and datalogger (Keithley 2400). The integrated systems were later operated in parallelly (I-P) connected and serially (I-S) connected closed-circuit modes to assess the overall power output. The polarization tests were performed to know the cell design point of the systems by using varying circuit resistance method at 200 KΩ to 4 Ω external resistances. The slope of the polarization curve represents the internal resistance of the system which was calculated using Eq. (1)

$$R_{int} = - dE/dI \text{ -----------eq (1)}$$

Where R_{int} is the internal resistance (Ohms), E represents potential (V), and I represents current (A).

When the reactors were operated in a closed-circuit mode, the current (I) was calculated using the Ohm's law i.e., $V = IR$

Power (mW) was derived from P=IV equation. Current density (mA/m^2) and power density (mW/m^2) were calculated by normalizing the respective absolute current and power values with the cathode surface area (m^2).

Chapter 3

Results and Discussion

3.1 Domestic sewage characterization

The DS collected from the STP facility at faculty residential complex (IISER-Mohali) was initially characterized for various pollutant concentrations. Table 1 represents the average influent pollutant concentrations collected before experiments or it was fed to the systems. A considerable amount of organic carbon (COD, 411±32 mg/L) and nutrients fractions (N & P) with near neutral pH were present in the influent.

Table 1. Influent domestic sewage characterization

Parameters	**Influent Domestic Sewage**
pH	7.5±0.05
Conductivity	1.06±0.1 mS
COD	411±32 mg/L
phosphates	3.5±0.3 mg/L
Ammonia	45±8 mg/L
Nitrates	0.9±1.1 mg/L
BOD	190±38 mg of O_2/L
Total coliforms	2.4×10^9 MPN/100ml

After initial characterization, the same DS was fed to the systems and effluent samples were analysed to access the removal of pollutants and to evaluate the performance of the systems as discussed in the following sub-sections.

3.2 Wastewater treatment performance of the systems

The consolidated data of various parameters analysed to evaluate the performance of two independent but identical systems (S1 and S2) at 3h HRT is presented in Table 2. The performance of these systems at different reactor conditions is discussed in terms of removal efficiencies for each parameter.

3.2.1 COD

The maximum COD removal efficiency of around 72% was achieved with the integrated system operated at different experimental conditions (I-O, I-P, and I-S), followed by C+E (64%), C (58%) and WC (20%) conditions at 3 h HRT (Fig. 3). It suggests that as the reactor components increased, the COD removal efficiency also increased. In particular, in the case of integrated system the presence of different microenvironments facilitated by different reactor components, viz., cocopeat, plants, and electrode assemblies led to the maximum COD removal. In WC experimental condition without any carrier material or other components, organic carbon removal occurs only through the aerobic microbial oxidation of organic matter present in the wastewater. In experimental condition C, i.e. with cocopeat, in addition to the aerobic treatment process, other processes like anaerobic treatment and physical adsorption of organic matter to the carrier material contribute to higher COD removal. High porosity nature and water holding capacity of cocopeat limit the air within the pores of the cocopeat and thus facilitates anaerobic microenvironment favoring the growth and activity of anaerobic bacteria. These microbes efficiently utilize the nutrients adsorbed by cocopeat. The C+E condition achieved additional COD removal most likely due to the activity of electrogenic microorganisms that can oxidize the organic matter by using anode as the terminal acceptor under anaerobic conditions. In the integrated system, in addition to the aerobic and anaerobic microbial processes, rhizosphere activity due to the presence of the plant enhanced the COD removal further. These results suggest that the multiple bioprocesses in a single system facilitate the utilization of complex organic matter at a faster rate through synergistic microbial interactions in different microenvironments.

Table 2. Consolidated data of different parameters of two independent but identical systems (S1 and S2) in all experimental conditions. (Inf: Influent, Eff: Effluent)

Experimental Conditions in the systems	System	Parameters								
		COD			Phosphate			Ammonia		
		Inf.	Eff.	% Removal	Inf.	Eff.	% Removal	Inf.	Eff.	% Removal
Without Cocopeat	**S1**	377±38	289±14	23	3.7±0.2	3.6±0.2	1.2	34±7	34±3	0.6
	S2	377±38	308±13	18	3.7±0.2	3.6±0.2	1.5	34±7	34±3	0.5
Cocopeat	**S1**	374±32	233±17	59	3.6±0.2	2.2±0.1	38	35±5	33±4	4.3
	S2	374±32	246±18	58	3.6±0.2	2.1±0.1	41	35±5	33±4	4.0
Cocopeat + Electrode	**S1**	409±37	133±15	65	3.1±0.3	0.8±0.1	73	55±8	38±5	28
	S2	409±37	145±14	62	3.1±0.3	0.8±0.1	72	55±8	37±6	25
Integrated (Open circuit)	**S1**	410±35	107±13	73	3.8±0.1	0.6±0.1	83	47±6	30±5	36
	S2	410±35	122±12	70	3.8±0.1	0.6±0.1	83	47±6	32±5	34
Integrated (Parallel circuit)	**S1**	453±34	140±15	68	3.3±0.2	0.7±0.1	79	51±5	35±4	32.5
	S2	453±34	138±13	69	3.3±0.2	0.8±0.1	75	51±5	35±3	31
Integrated (Series circuit)	**S1**	442±37	130±14	70	3.4±0.3	0.7±0.1	78	44±4	30±4	32
	S2	442±37	118±14	73	3.4±0.3	0.9±0.1	74	44±4	30±5	31

Experimental Conditions	System	Parameters						
		BOD			pH		Nitrate	
		Inf.	Eff.	% Removal	Inf.	Eff.	Inf.	Eff.
Without Cocopeat	**S1**	160±0	150±0	7	7.41±0.1	7.85±0.04	1.41±0.01	1.52±0.5
	S2	160±0	150±0	7	7.41±1	7.82±0.2	1.41±0.01	1.56±0.3
Cocopeat	**S1**	165±5	65±5	61	7.44±0.1	7.83±0.03	0.0	0.66±0.3
	S2	165±5	65±5	61	7.44±0.1	7.84±0.03	0.0	0.75±0.4
Cocopeat + Electrode	**S1**	160±10	40±0	75	7.47±0.07	7.84±0.02	0.0	5.9±0.3
	S2	160±10	40±0	75	7.47±0.07	7.83±0.04	0.0	5.8±0.4
Integrated (Open circuit)	**S1**	175±15	30±10	83	7.51±0.05	7.87±0.03	0.28±0.13	5.5±0.5
	S2	175±15	35±5	80	7.51±0.05	7.88±0.02	0.28±0.13	5.4±0.5
Integrated (Parallel circuit)	**S1**	245±25	80±10	68	7.53±0.05	7.83±0.05	0.0	3±0.3
	S2	245±25	70±10	72	7.53±0.05	7.85±0.04	0.0	4.3±0.4
Integrated (Series circuit)	**S1**	230±20	60±10	74	7.52±0.06	7.86±0.04	3.9±0.3	6.1±0.3
	S2	230±20	50±10	79	7.52±0.06	7.9±0.02	3.9±0.3	6.3±0.4

Notes: Without cocopeat (WC), with copeat (C);, with cocopeat and electrodes (C+ E), and Integrated system has all components (Cocopeat, electrodes and plant)

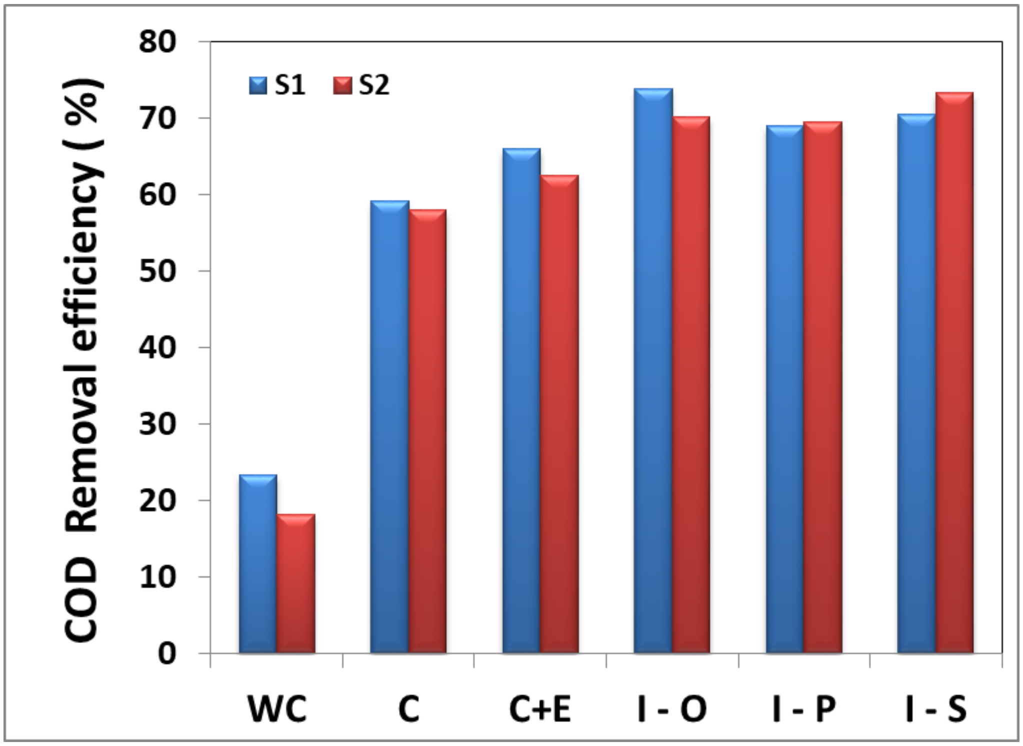


Figure 3. COD removal efficiency of two independent but identical systems (S1 and S2) at different experimental conditions (WC: without cocopeat, C: cocopeat, C+E: cocopeat with electrode, I-O: integrated open circuit, I-P: integrated parallal circuit, and I-S: integrated series circuit).

3.2.2 Phosphate

The phosphate contamination in sewage occurs mainly due to the anthropogenic activity (cleaning and washing activities). The presence of phosphates at higher concentration in effluents leads to eutrophication of the water bodies. Hence, their removal from the wastewaters is desired. Phosphates are available in three forms, a) organic phosphorous (associated with organic molecules), b) orthophosphate and c) polyphosphates. Orthophosphate can be precipitated through chemical methodology. While organic phosphorous and polyphosphates can be converted to orthophosphate through a biological process. A negligible amount of phosphates was removed (1.3%) in control (WC) experimental condition which can be attributed to the absence of specific microbial activity or because of the phosphate presence in the oxidized form which does not get removed in the aerobic environment (fig 4). This is well in agreement with the fact that only aerobic processes does not lead to the removal of phosphates from the wastewater by phosphate-accumulating microorganisms (PAOs). They need alternate aerobic and anaerobic zones (Ubukata, 2006). Irrespective of the electric circuit mode operation (I-O, I-P or I-S), the maximum phosphates removal efficiency of about 80% was observed with the integrated systems. It can be attributed to the efficient uptake of phosphates by the plants and PAOs. Whereas, experimental condition C (40%) showed comparatively much lesser phosphate removal than the C+E (72%) as well as integrated systems. The phosphate removal efficiency is higher in C+E than C condition because in C+E condition, electrocoagulation of negatively charged phosphates at positive anode can occur along with the activity of PAOs (Tian et al. 2017).

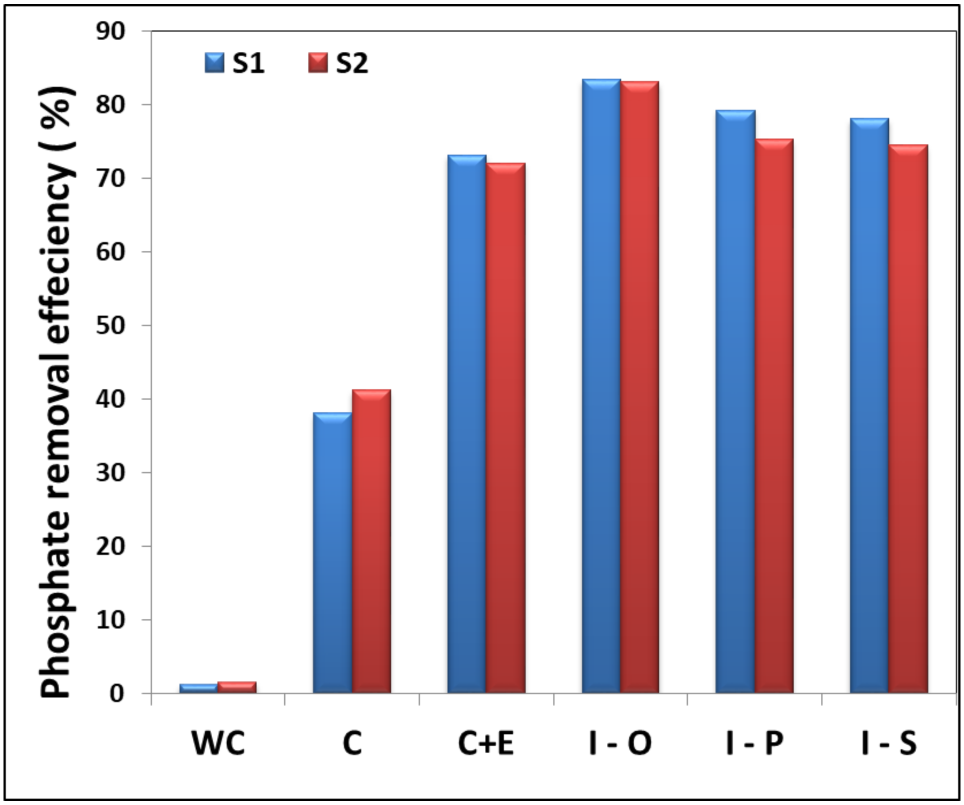


Figure 4. Phosphate removal efficiency of two systems at different experimental conditions

3.2.3 Nitrates and Ammonia removal

Excess usage of detergents and cleaning agents (containing high N concentration) in addition to the urine contribute to the water pollution through the addition of high nitrate or ammonia concentrations. The influent domestic sewage collected from the settling tank of STP contained high ammonia and low nitrate concentrations (45±8 mg/L and 0.9±1.1 mg/L). Ammonia removal followed a similar trend as that of phosphate removal in the systems operated at different experimental condition at 3 h HRT. Irrespective of the electric circuit connection mode (I-O, I-P, and I-S), the integrated systems showed maximum ammonia removal efficiency of 35%, followed by C+E (26%), C (4%) and WC (0.5%) systems (Fig. 5).

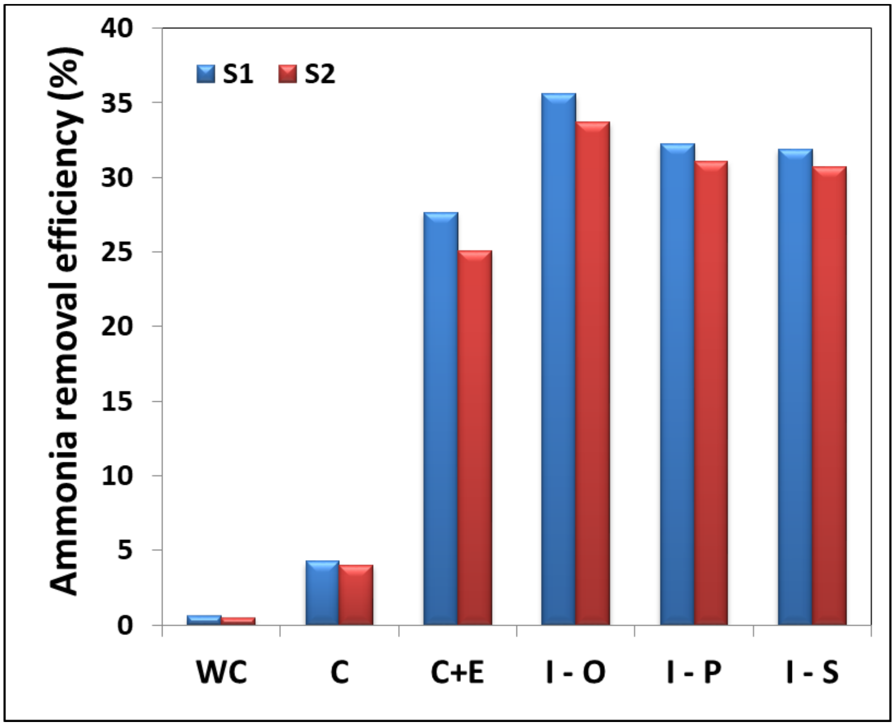


Figure 5. Ammonia removal efficiency at different experimental conditions.

Ammonium is less mobile than nitrate and biologically more available form of inorganic nitrogen which can be easily assimilated into microbial and plant biomass. These are the most plausible reasons of maximum ammonia removal in the case of integrated systems. Even though the enzymatic activity of nitrate and nitrite reductase in microbes is not inhibited in the aerobic environment of WC operated condition, less ammonia removal efficiency was observed. This could be due to the suppression of the enzymatic activity by free ammonia present in the influent feed (Singh, 2013).The absence of cocopeat to facilitate multiple microenvironments within the system might also have hindered the ammonia removal in WC experiment condition. The ammonia removal efficiency is higher in C+E than C condition. This could be because of microbial ammonia oxidation process at the anode thereby contributing to removal efficiency. In addition the processes like nitrification and denitrifictaion can occurr due to availability of multiple microenvironments in cocopeat (Wang et al. 2012); (The Water Planet Company, 2016).

A significant increase in the nitrate concentration was observed in the effluents in all cases of reactor conditions (WC: 1.41±0.01 to 1.54±0.03 mg/L; C: 0 to 0.7±0.06 mg/L; C+E: 0 to 5.85±0.12 mg/L; I-O: 0.28±0.13 to 5.45±0.08 mg/L; I-P: 0 to 3.66±0.8 mg/L; I-S: 3.9±0.31 to 6.2±0.2 mg/L) (Fig 6). This is most likely due to the nitrification process that results in conversion of ammonia to nitrate (The Water Planet Company, 2016).

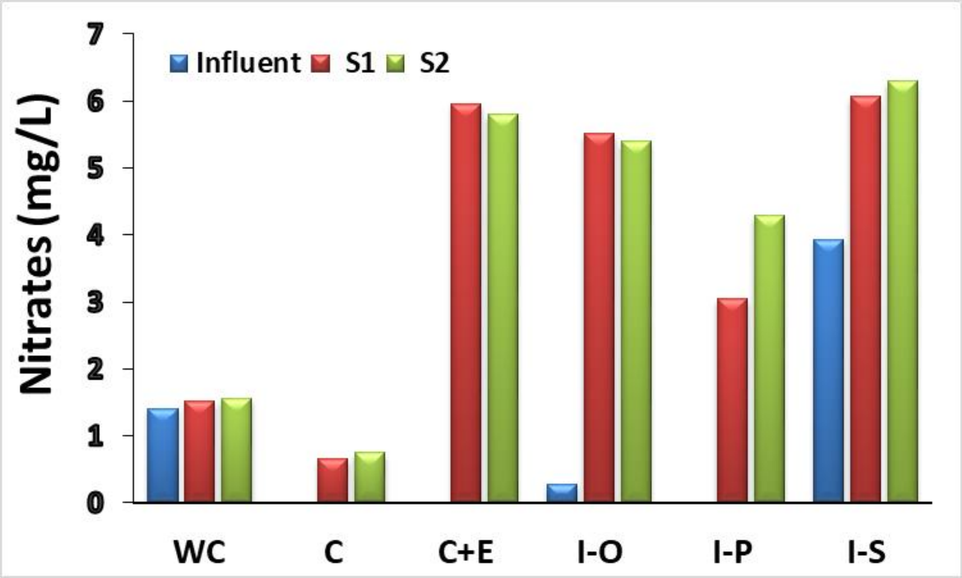


Figure 6. Changes in nitrate concentration of two systems at different experimental conditions.

3.2.4 pH

Determination of pH plays a vital role in the wastewater treatment process as pH has a direct influence on wastewater treatability regardless of whether treatment is physical, chemical or biological. Abnormal or irregular pH in biological treatment processes can result in a significant decrease in the rate of removal of organic compounds from the environment. Therefore, pH of both influent and effluent has to be monitired. The effluent results show increase in pH in all experimental conditions (Fig 7). The reason for this might be the stripping of CO_2 formed because of organic matter degradation (Cohen and Kirchmann, 2004). The overall effluent pH was 7.85 ±0.03, which did not affect the biological treatment of wastewater.

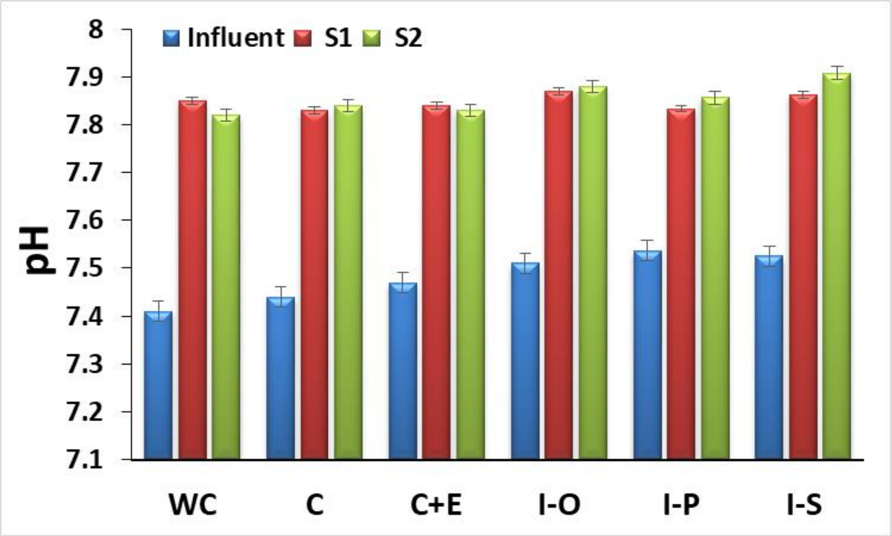


Figure 7. Increase in effluent pH of both systems at different experimental conditions.

3.2.5 Total coliforms

Total coliforms, particularly fecal coliform bacteria are found in wastewater if it is contaminated with the fecal waste of human or animal origin. Fecal coliforms are primarily used to indicate the presence of pathogenic bacteria such as *Salmonella spp.*, *Vibrio cholerae*, and pathogenic *E. coli*. MPN test was conducted to quantify the total coliforms count. Average total coliforms in the influent were estimated to be $2.4\times10^9\pm1.5\times10^1$ MPN/100ml. There was no significant change in coliforms count in all the experimental conditions except C, in which ten-fold decrement in total coliforms was sobserved. These results suggest that the proposed system is not effective for the removal of coliforms from the domestic sewage. An additional disinfection treatment of the effluent is therefore needed to remove coliforms.

3.2.6 Biological Oxygen Demand (BOD_5)

The BOD indicates the amount of oxygen needed to decompose organic compounds in wastewater by microbial activity. For the determination of $cBOD_5$, nitrification inhibitors were added to the samples, which suppress the degradation of nitrogen compounds. Consequently, it results in the determination of the decomposition of carbon compounds only (cBOD). From the data, we observed that the trend of removal efficiencies of both the cBOD and COD are the same in all experimental conditions. The maximum BOD removal efficiency of around 77% was achieved with the integrated system (I-O, I-P, and I-S), followed by C+E (75%), C (61%) and WC (7%) systems at 3 h HRT.

3.2.7 Electrical Conductivity

Electrical Conductivity is the measure of the ease with which wastewater conducts electricity. It is an important parameter in the case of microbial electrochemical technologies such as microbial fuel cells, one of the important component of the integrated system. It also indicates the total dissolved salt (TDS) content of the water. Average influent conductivity was 1.06±0.1 mS and after 3 h operation, it remained the same in all experimental conditions.

3.3 Wastewater treatment evaluation at the longer Hydraulic Retention Times (HRTs)

The integrated systems were operated at extended HRTs of 6 and 12 h to assess the maximum wastewater treatment efficiency. Samples were collected at different HRTs and analyzed for various parameters. As expected, improved performance in terms of pollutant removal was observed at the longer HRTs (Table 3). At an HRT of 12 h, more than 85% removal in COD and phosphate was observed due to a maximum exposure of wastewater to microbes and plants present in the system. The ammonia removal was also more than 75 % at 12 h HRT. These results clearly suggest that the integrated system can achieve efficient removal of the major pollutants from wastewater.

Table 3: Wastewater treatment at longer HRTs

Parameters	**Influent**	**HRTs**		
		3 h	**6 h**	**12 h**
pH	7.6±0.02	7.9±0.03	7.95±0.03	7.92±0.03
COD (mg/L); (%)	455±28	105±26; (77)	75±22; (83)	54±15; (88)
Phosphates (mg/L); (%)	3.3±0.3	0.77±0.3; (76)	0.6±0.4; (80)	0.5±0.2; (85)
Ammonia (mg/L); (%)	51±8	33±6; (35)	24±5; (52)	12±3; (76)
Nitrate* (mg/L); (%)	2±0.3	5.3±0.2; (62)	6.1±0.2; (66)	7.1±0.2; (71)

Notes: The values in parentheses represent % removal efficiency of each parameter.*For nitrate, the values in parantheses indicate the increase in its cocnentration due to nitrofictaion activity.

3.4 Plant growth

C. citratus had shown considerable growth in terms of leafy biomass (nearly 45±15cm long leaf blades) after one month of its plantation in the system (fig 13). The continuous feeding of DS to the system ensured the availability of nutrients for the plants in each reactor unit. The dry weight of biomass per plant was approximately 216±39 mg. Apart from the uptake of nutrients from DS, plants also sequester the atmospheric as well as CO_2 generated from microbial degradation of organic carbon and fix it in the form of biomass. This is an additional advantage of the integration of plant component in the system. It can help to make the wastewater treatment processes CO_2 neutral or positive.

Photograph 6. Photographical representation showing the growth of plants after 1 month

3.5 Bio-electrogenic activity and energy recovery

The activities of electrogenic microorganisms growing at the anode and cathodic oxygen reduction were monitored by measuring OCV of the system. The performance of the integrated system was further evaluated by connecting the individual MFC units in both series and parallel modes.

3.5.1 Open Circuit Voltage

Electroactive microorganisms growing at the anode oxidize organic carbon (present in wastewater and root exudates) and release electrons that can be harvested in the form of electricity. The potential developed between two unconnected electrodes (anode and cathode) of the system was measured as the OCV**.** The bio-electrogenic activity in terms of OCV was monitored after accommodating the electrode assembly into the system (C+E). The average OCV profiles of the two systems are shown in Figure 14. When the systems were operated without plants, maximum OCV recorded was around 0.188±0.010 V, whereas, increased OCV (0.22±0.010 V) was observed after planting *C. citratus* in both systems (Fig 8). In the absence of plants, the system mimics the operation of Microbial Fuel Cell (MFC). In MFC, microbes extract electrons from organic substrates present in wastewater by their metabolism and transfer the electrons (directly or indirectly) to the anode. These electrons are then transferred to the cathode via external circuit and are consumed in oxygen reduction reaction. By placing appropriate load in the external circuit, electricity can be harvested. The plant insertion into the system mimics plant microbial fuel cell (P-MFC) operation. P-MFC is a promising modification of MFC that is based on the unique plant-microbe relationship in the rhizosphere region of a plant (Nitisoravut and Regmi, 2017). Plants grow by nutrient uptake and fix CO_2 in the form of carbohydrates. The fixed carbon compounds translocate to the roots of the plants and released in the form of exudates. These exudates or rhizodeposition of the plant provides the additional supplementation of the carbon required for the propagation of microbial consortia. The rhizosphere mediated electrogenesis by placing electrode assembly in the rhizosphere further aids the electric power generation (Chiranjeevi et al., 2012). Thus the increase in OCV in the case of integrated systems can be attributed to the presence of root exudates and rhizosphere activity.

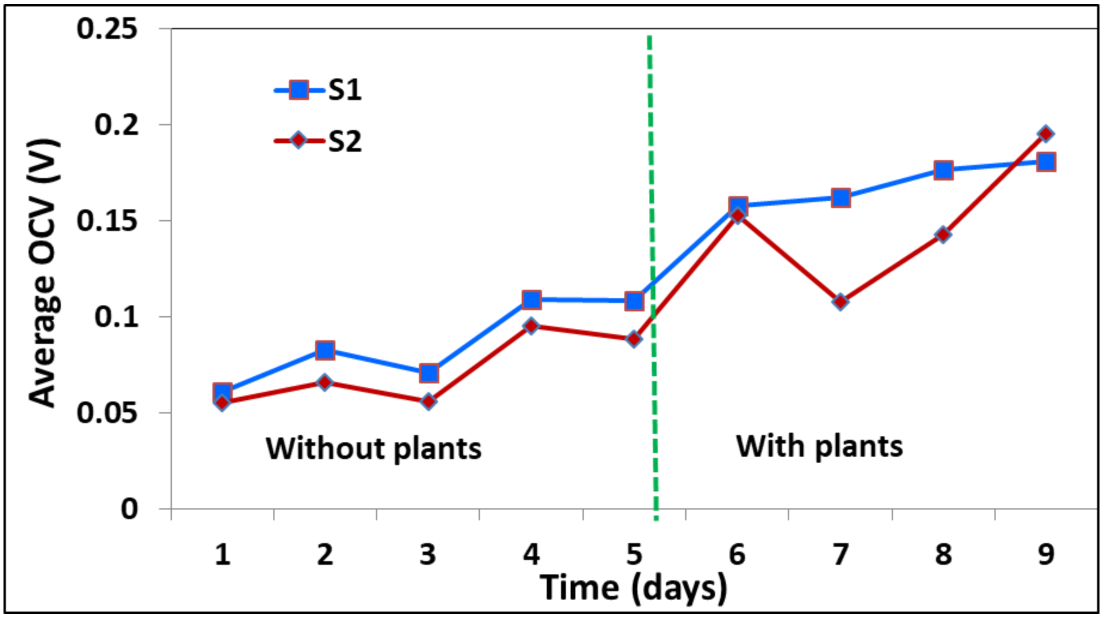


Figure 8. Bio-electrogenic activity of two independently operated systems (S1 and S2) in terms of open circuit voltage (OCV) at different experimental conditions (C+E and I conditions).

3.5.2 Polarization and power density curves of the integrated systems

Polarization curve is a powerful tool for the analysis and characterization of microbial fuel cells. It expresses the voltage as a function of current density. Polarization tests were conducted by connecting individual MFC units in parallell and serial modes to determine the cell design point that is critical to harvest usable electric power from the whole system. The power density curves were also plotted to determine the maximum power point. Polarization behavior of closed, integrated circuits was recorded during the stable phase of operation using 200–0.004k ohm resistors. Electric current output showed a decreasing trend with an increase in the resistance. Low resistance allows more e− flow in the fuel cell circuit, and this results in the potential drop, especially at lower resistances despite higher current density. At maximum load (200 k ohm), negligible current output was recorded. Maximum power densities of 30.9 mW/m^2 and 31.6 mW/m^2 were observed in serially connected system at 20 k and in a parallel connected systems at 0.180 k ohms, respectively (Fig 9). The point at which maximum power density is observed in the power curve against the current density is considered as a cell design point of that particular fuel cell system (Venkata Mohan, Veer Raghavulu, et al., 2008). Active power output can be obtained on the left side (low current density region) of the cell design point.

The systems were operated for a week in series connection to evaluate a continuous and long-term power generation, and it showed continuous stable power density generation of 0.13±0.02and 0.03±0.01 mW/m^2 for high and low COD strength DS respectively. These results suggest that the integrated system can generate low levels of power output. With further improvements in the reactor design, the use of conductive materials as the carrier material, operational conditions and efficient oxygen reduction catalysts, a considerable improvement in power outputs is expected.

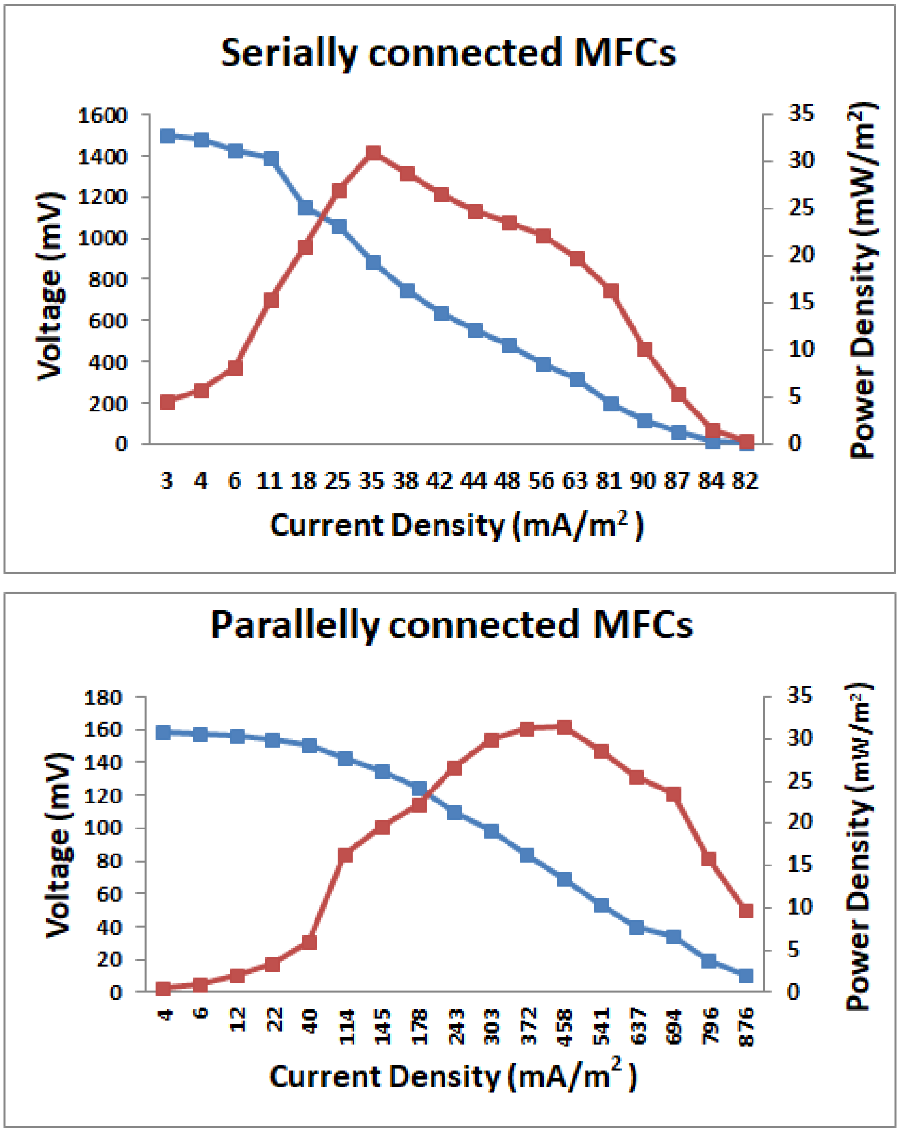


Figure 9. Polarization and Power density Curves of S1 (a. serially connected MFCs, b. parallaly connected MFCs).

Chapter 4

Conclusions and future prospectives

The integrated hydroponics-microbial electrochemical technology platform showed efficient domestic wastewater treatment with simultaneous electricity generation and commercially important plant cultivation. The novelty of the proposed system lies on the integration of various processes, viz., aerobic, anaerobic, hydroponics, and microbial electrochemical technology in one system (Fig 10).

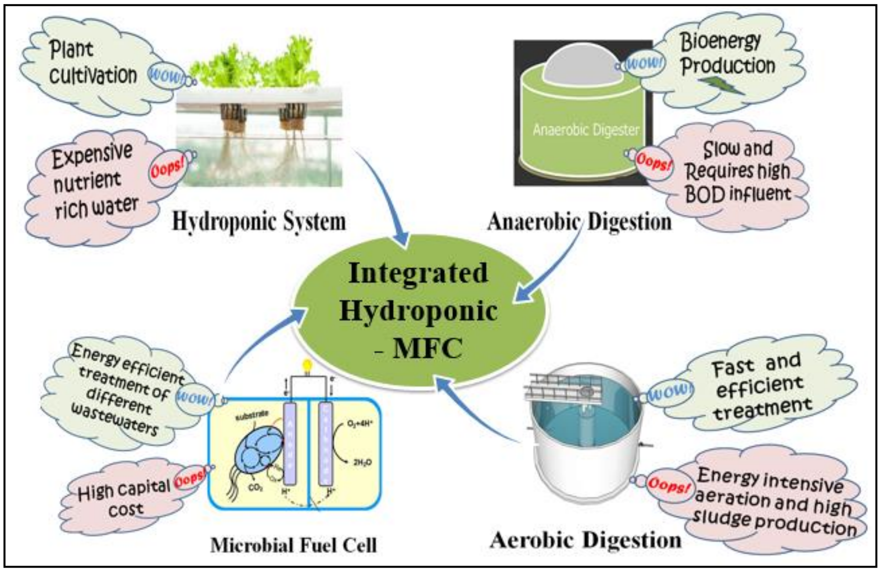


Figure 10. Integrated Hydroponics-Microbial electrochemical technology platform based on the existing plant cultivation and wastewater treatment approaches.

It allowed the exploitation of multiple bioprocesses, viz., aerobic, anaerobic, rhizospheric, electrogenic and plant roots along with the abiotic components not only for the efficient removal of different pollutants from DS, but it also offered simultaneous recovery of electricity, and commercially valuable plant biomass (Fig 11). Cultivation of plants through the proposed approach allows both the removal and recovery of nutrients such as N & P in the form of biomass. The CO_2 uptake by plants makes it a CO_2 neutral or positive wastewater treatment technology. The innovative design of arranging and operating the smaller treatment units seperately is likely to allow troubleshooting and addressing the maintenance issues of the system efficiently without compromising on the continuous wastewater treatment process. It is the major advantage over the existing centralized as well as decentralized wastewater treatment technologies that are mostly based on the use one or two big reactor units. This proof-of-principle study also underscores the importance of creating different microenvironments and thereby facilitating different biological activities within a single system for efficient decentralized wastewater treatment and resource recovery.

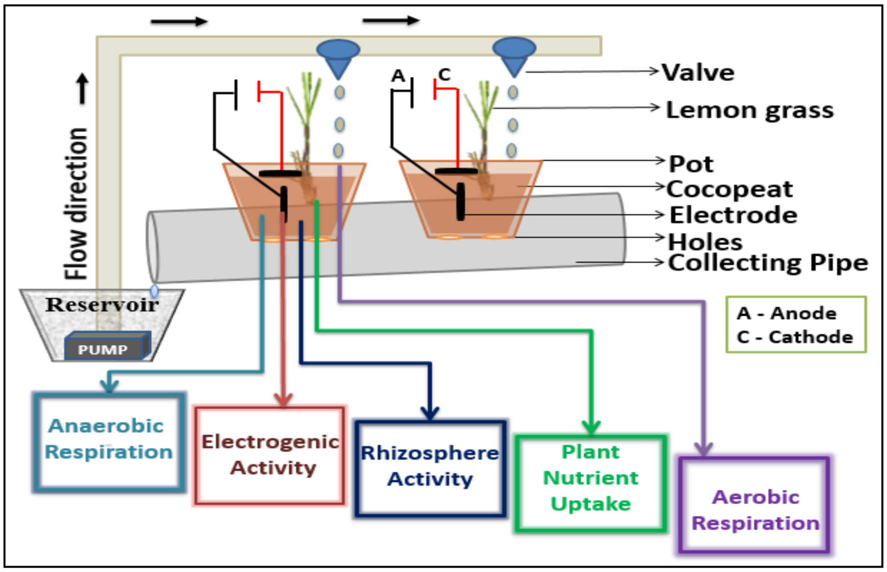


Figure 11. Schematic of the Integrated System showing various biological processes that can occur in each reactor unit.

The treatment efficiencies and energy recovery can be further enhanced through the optimization of reactor as well as process parameters. Reactor parameters include, e.g., the width to depth ratio of individual reactor units, conductive carrier/support bed materials, catalysed electrode materials, and plants with better growth rates and nutrient uptake capabilities. Further work will be focused on designing and optimizing a larger scale system of 1 m^3 wastewater treatment capacity and understanding the complex biological interactions occurring in various microenvironments involved in the removal of different pollutants from the wastewater. The removal of emerging pollutants such as heavy metals, microplastics, pharmaceutical residues, etc. will also be evaluated in the future studies.

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