Sewage enhanced bioelectrochemical degradation of petroleum hydrocarbons in soil environment through bioelectro-stimulation

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ABSTRACT

The impact of readily biodegradable substrates (sewage and acetate) in bioelectroremediation of hydrocarbons (PW) was evaluated in a bench-scale soil-based hybrid bioelectrochemical system. Addition of bioelectro-stimulants evidenced efficient degradation than control operation. Acetate and sewage were exhibited power density of 1126 mW/m^2 and 1145 mW/m^2, respectively, which is almost 15 % higher than control (without stimulant, 974 mW/m^2). Increased electrochemical activity was correlated well with total petroleum hydrocarbons (TPH) degradation through addition of acetate (TPH$_R$, 525 mg/L, 67.4 %) and sewage (TPH$_R$, 560 mg/L, 71.8 %) compared to the control operation (TPH$_R$, 503 mg/L, 64.5 %). Similarly, chemical oxygen demand (COD) reduction was also enhanced from 69.0 % (control) to 72.1 % and 74.6 % with acetate and sewage, respectively. Sewage and acetate also showed a positive role in sulfates removal, which enhanced from 56.0 % (control) to 62.9 % (acetate) and 72.6 % (sewage). This study signifies the superior function of sewage as biostimulant compared to acetate for the bioelectroremediation of hydrocarbons in contaminated soils.

1. Introduction

Contamination of soil by petroleum-based hydrocarbons is one of the critical environmental issues. Petroleum shares a major part among energy sources that uphold the economic and social development of a nation. Exploration, transportation and processing of petroleum and petroleum products adversely affect the soil environments and exert severe damage in the soil ecosystem [1,2]. Accidental release of crude oil into the environment is also one of the major reasons for soil contamination [3]. Petroleum hydrocarbons biologically degrade over time. Microbes and plants were found to involve in the bioremediation of such petroleum-contaminated soils [4]. The complexity associated with polycyclic aromatic hydrocarbons causes slower degradation rates and decreases the bioremediation efficiency [5]. Poor bioavailability and long degradation periods are the major challenges for petroleum contaminated soils bioremediation [6]. Several

physicochemical techniques such as thermal desorption, washing, chemical oxidation and electrochemical oxidation were evaluated to reduce the remediation time [3]. Bioelectrochemical systems or microbial fuel cells found to exhibit their efficiency towards treatment of various wastewaters and pollutants [7,8]. The microbial communities of bioelectrochemical systems (BES) were found to be diverse and showed effective performance towards bioremediation of petroleum-contaminated soils [9–12]. Bioelectroremediation through BES attracted attention due to sustainability in terms of energy and the use of microorganisms as catalyst to promote the redox reactions [13,14]. Compared to conventional biological treatment of recalcitrant and complex organic molecules, BES found to exhibit superior performance towards treatment which was termed as bioelectrochemical treatment (BET) [15].

Soil bioelectroremediation was found to exhibit-limited efficiency due to the complexity of hydrocarbons and lack of substrates that are favorable for efficient microbial activity. Addition of biodegradation enhancers or biostimulants is considered as the most efficient method for soil bioremediation [16,17]. The addition of growth-limiting nutrients such as nitrogen, phosphate and organic carbon improves bioremediation of the contaminated soils [18,19]. Supplementation of nutrients enhances the metabolic activity of the indigenous microbial community, and this is called as biostimulation [20]. Several agents such as urea, plant residues, composted plant biomass, meat and bone

meal, crop residues from corn and sugarcane, *etc.*, were also used to enhance soil bioremediation [21–23]. The strategy of biostimulation was also found to be effective in BES towards electricity generation from microbial fuel cells (MFCs) [24,25] and heterotrophic-autotrophic denitrification [26]. BES were proved to act efficiently with vast varieties of substrates [27–29]. Similarly, the BES were also evidenced as a promising route for the treatment of hydrocarbon contaminated soils, biostimulation strategy can be applied to improve hydrocarbons degradation efficiency in the soil environment [30]. The hybrid microbial electrochemical system that has bioanodic oxidation and biocathodic reduction functions in soil environment was reported to treat petroleum refinery wastewater (PRW) under applied potentials in the range of 0.5–2.0 V [30]. The study evidenced a promising degree of degradation concerning to chemical oxygen demand (COD, 69.2 %) and TPH (90 %) over 7 days of operation under 2 V of applied potential. In a soil BES that operated to treat contaminants from real-field petroleum refinery wastewater (PRW). Efficient degradation was achieved for diesel range organics (DROs). DROs with higher carbon number such as n-Hexadecane, n-Octadecane and n-Eicosene were degraded by 80 %. Simple DROs such as n-Decane and Surrogate were found to degrade completely [30]. In another study with soil based MFC using simulated PW, about 50 % of the DROs removed. Whereas, n-Decane increased its concentration. The improvement in n-Decane concentration was attributed to incomplete bioelectrochemical remediation of higher DRO molecules [31].

In this direction, a novel system was evaluated for enhancement in bioelectroremediation of hydrocarbon contaminated soils by using hybrid bioelectrochemical system in soil microenvironment that function both anodic and cathodic reactions bioelectrochemically. Further, sewage and acetate were used as enhancers for bioelectrochemical activity compared with control operation. Hybrid soil-based BES consists of bioanode and biocathode for efficient degradation of hydrocarbon compounds in soil microenvironment. The present study was designed for bioelectrochemical treatment of soil pollution due to PW contamination. In a previous study, PRW used as substrate using hybrid soil-based bioelectrochemical system, about 51 % of TPH and 69 % of COD were removed successfully [26]. The major differences in nature and composition of PW and PRW are TDS, COD and TPH concentrations. High TDS pertained to PW exhibits distinct bioelectrochemical properties. Further, supplementing a simple organic substrate (raw sewage and acetate) to soil contaminated with PW was evaluated to identify its function on degradation as well as bioelectrochemical properties. Critical attention was also given to identify the specific influence of acetate and sewage on bioelectrochemical activity during the treatment of PW.

2. Materials and methods

2.1. Design of soil based microbial electrochemical reactor

Soil based bioelectrochemical system that contains bioanode and biocathode was developed initially to evaluate the function of external supply of electrochemical potential on degradation of petroleum-based pollutants. A single chambered membrane-less and soil-based microbial electrochemical reactor having total volume of 1.13 L was used for the study [26]. The reactor was made of acrylic glass in rectangular shape, having height of 14 cm and width of 9 cm (Fig. 1a). Mixture of sand and peat moss was added in the ratio of 70 and 30 to the reactor. Soil and peat moss exhibited void ratio of 0.6 with water holding capacity of 0.49 L in the total working volume of 0.81 L. Plain graphite based plates having total surface area of 94 cm^2 (length, 7 cm; breadth, 5 cm; height 1 cm) were used as electrodes for anode and cathode. The electrodes were placed vertically in the soil column. Anode and cathode electrodes that were developed with respective biofilms in a suspended MFC reactor were transferred carefully to the soil system and evaluated the influence of external power supply on degradation of PW. Equal distances were maintained from the wall and each electrode. Non-corrosive titanium wire (0.4 mm diameter) was used to connect the electrodes and the external connections with resister and multimeter. Peristaltic pump was used for recirculation of water from the reservoir to the soil based electrochemical reactor. Inlet connection was placed in the side wall of the reactor (2 cm from the bottom) to provide up flow in the soil column. Another connector from the other end of the reactor (6 cm from the top) from the top of the side wall was used as an outlet by gravity flow to the reservoir. The water was recirculated through the reactor for 7 days of operation in each batch cycle.

2.2. Wastewaters used in the study

The present study used two types of wastewaters *viz.*, simulated PW [32] and raw sewage. In addition, PW was also used along with acetate. The composition of simulated PW (all the compositions in g/L, NH_4Cl, 0.25; $FeSO_4$, 0.25; $CaCl_2.2H_2O$, 15.0; KCl, 2.0; $MgCl_2$, 15.0; NaCl, 55.0; Na_2SO_4, 2.0; $NaHCO_3$, 1.0; H_3BO_3, 0.25) was followed from previous studies [31]. The original PW was diluted for 12 times to bring the TDS and TPH concentrations to 780 mg/L and 7800 mg/L, respectively. Raw sewage (COD, 320 mg/L; TDS, 1700 mg/L, nitrates, 12.4 mg/L; sulfates, 600 mg/L, pH, 7.99) was collected from North Doha sewage treatment plant. After grit removal, grab sampling method was used to collect the sewage. During evaluation of acetate as biostimulant, 410 mg of acetate (equivalent of 320 mg/L) was added to each liter of PW. This helped to evaluate both substrates as biostimulant under similar COD concentrations.

2.3. Operation

The major objective of the present study was to evaluate sewage and acetate as stimulants for *in situ* BET of hydrocarbons contaminated soils. Sewage and acetate were used as substrates to enhance bioelectrochemical activity that in turn enhance bioelectroremediation in soil microenvironment. Simulated PW was diluted to lower the concentrations of TPH and TDS to 780 mg/L and 7800 mg/L, respectively. The complete study was performed in three phases as shown in Fig. 1b. Based on the optimization studies performed using petroleum refinery wastewater in a similar reactor, 2.0 V was considered to operate the present study. DC power supply unit was used for continuous voltage supply in the soil BES [30]. Recirculation of the PW in up-flow pattern from the reservoir to the reactor was carried out at a rate of 30 mL/h. Batch mode operation with operating cycles of 7 days in each cycle was considered. After stable operation with respect to substrate degradation was identified in the soil BES, five consecutive cycles were operated with PW (Phase 1). The performance of soil BES was evaluated in terms of reduction of TPH and COD along with current density evolution during experimentation. Subsequently, feed was changed to PW along with acetate and the soil BES system was operated for 3 cycles (Phase 2). Later, soil BES was operated with PW and raw sewage for three cycles (Phase 3) (Fig. 1b). Prior to shifting the feed from PW + acetate to PW + sewage (between Phase 2 and 3), PW was recirculated through the reactor for 3 days to bring the system to the conditions of phase 1 (control phase) of the study. This helps to eliminate the acetate traces in the soil column and to compare the accurate influence of stimulants on BET. During all the three phases of the soil BES operation, the TPH of the wastewaters was kept constant. Inlet and outlet samples from the three phases of the reactor operation were collected and stored at 4 °C for analyses.
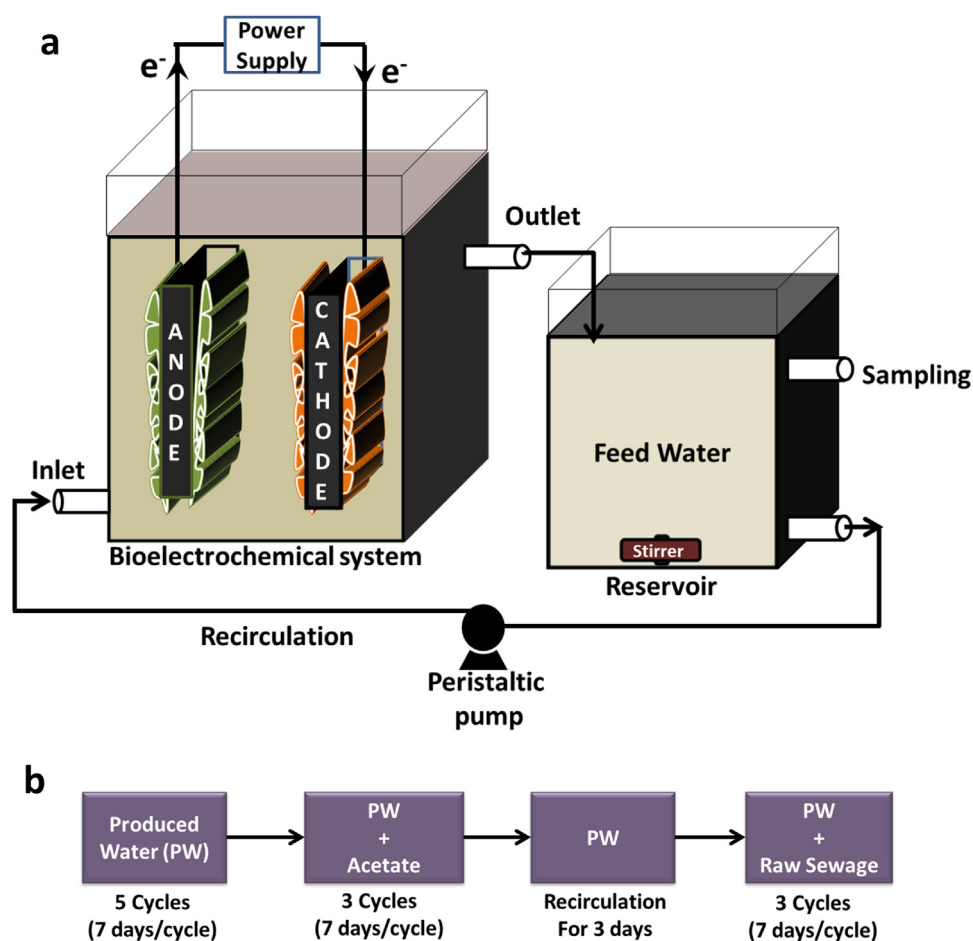
Fig. 1. (a) Schematic representation of bioelectrochemical system (BES) with bioanode and biocathode configuration for the bioremediation of petroleum hydrocarbon contaminants in soil. The system was also evaluated with acetate and sewage along with produced water for enhanced bio-electroremediation. (b) Sequence of operations with different biostimulants used in the study.

2.4. Analysis

During the operation of soil BES in the three phases, collected samples were analyzed for several parameters such as COD, TPH, sulfates, TDS and pH. All the analyses were followed the methodology outlined in the Standard methods of analysis [33]. COD was measured by LANGE COD testing kit, UK. Prior to analysis, all the samples were brought to room temperature. Current generation during BES operation was recorded with digital multimeter. Current density and power density were calculated by normalizing with surface area of electrode.

3. Results and discussion

3.1. Soil BES performance for produced water treatment

In this study, soil BES system operated in simulated field conditions showed potential in treating petroleum hydrocarbon pollutants from PW. COD and TPH removal (TPH$_R$) recorded from the soil BES evidenced bioanodic oxidation and concomitant biocathodic reduction activity for treatment of PW. From the five cycles of operation in the first phase, TPH$_R$ was found to exhibit more than 60 % efficiency after 7 days using 2 V of applied potential (Fig. 2a). During the second cycle of operation, the initial concentration of TPH (780 mg/L) was reduced to 243 mg/L accounting for degradation of 537 mg/L and efficiency of 68.85 %. From the five cycles of operation, the average value of TPH$_R$ was registered as 503 mg/L (64.5 %) (Table 1). COD that comprises all

the organic matters present in PW was also reduced from the operation of the soil BES (Fig. 3a). Untreated PW that found to exhibit 1020 mg/L of COD was reduced to 305 mg/L concentration. This was contributing to the maximum COD reduction of 720 mg/L (70.59 %) during the first operating cycle (Fig. 3a). The average COD removal from the five cycles of operation was registered as 704 mg/L (69 %) (Table 1). Both TPH and COD were analyzed at different time intervals, which showed a typical pattern of biological degradation of batch mode operation. Inlet TPH concentration of 780 mg/L was gradually decreased with time and reached to 295 mg/L by the end of the cycle (7 days) recording 69.5 mg/L-day of TPH removal rate (Fig. 2b). PW showed maximum TPH degradation rate of 80 mg/L-day during 48 h of operation. Similar to TPH$_R$ pattern, COD reduction was also evaluated in a single cycle of operation which also exhibited clear correlation with TPH$_R$. A maximum COD reduction rate of 109 mg/L-day was recorded under bioelectrochemical degradation of PW in soil matrix (Fig. 3b).

Several studies were focused on the bio-electroremediation of soil contaminated with petroleum-based pollutants through BES. BESs are known for enhancing the remediation of hydrocarbon-contaminated soils compared with natural attenuation. Anaerobic degradation facilitated by electroactive bacteria using electrodes as an electron acceptor is the primary mechanism for degradation of hydrocarbons [34]. Apart from the anaerobic mechanism, aerobic hydrocarbon-degrading bacteria such as *Parvibaculum* and *Pseudomonas* were also found to be involved in hydrocarbon degradation in soil [34]. The reactor design used for the present study facilitates both anaerobic and aerobic microenvironments, which
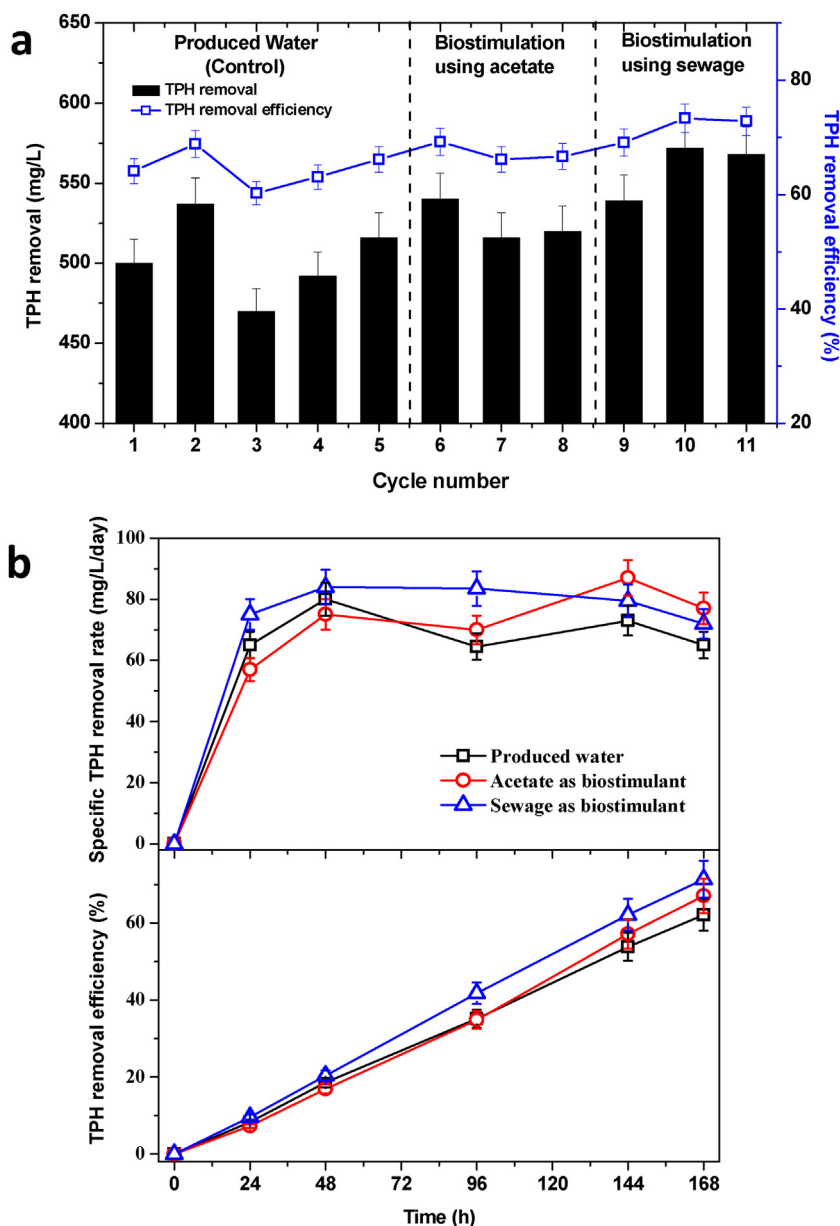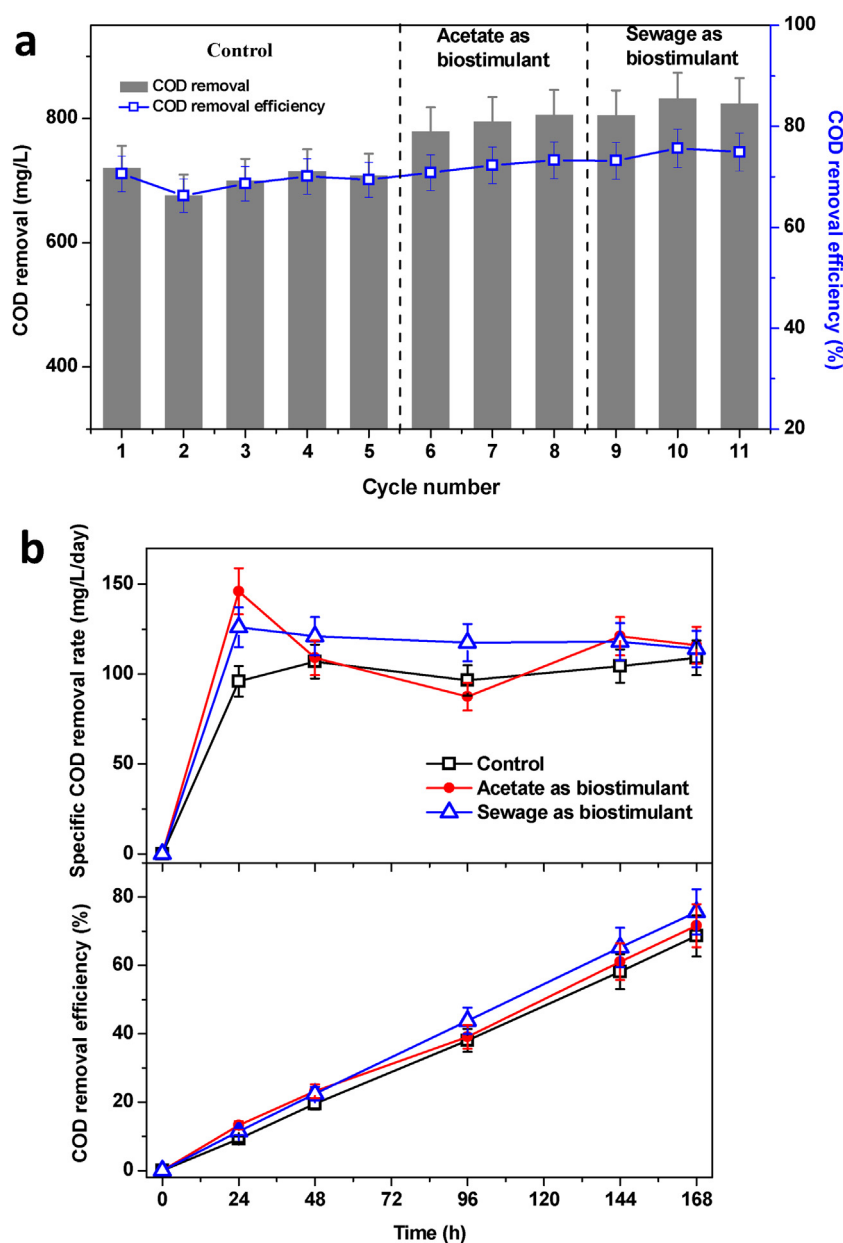
Fig. 2. Degradation of total petroleum hydrocarbons (TPH) of PW through BES under the influence of acetate and sewage in comparison with the control operation (a) Cycle wise performance and (b) Hour wise performance in a single operating cycle.

Table 1
Consolidated representation of biostimulation function on the bio-electrochemical degradation of petroleum based pollutants in bench-scale BES system (average values from each operational variation were considered herewith).

Parameter	Units	Control	Acetate as biostimulant	Enhancement over control	Sewage as biostimulant	Enhancement over control
Current density	**mA/m²**	479 ± 12	552 ± 9	73	567 ± 5	88
Power density	**mW/m²**	957 ± 25	1105 ± 19	148	1133 ± 10	176
TPH removal	**mg/L**	503 ± 25	525 ± 13	22	560 ± 18	57
	%	64.5 ± 3.2	67.4 ± 1.6	2.8	71.8 ± 2.3	7.3
COD removal	**mg/L**	704 ± 17	793 ± 14	89	820 ± 14	117
	%	69.0 ± 1.7	72.1 ± 1.2	3.1	74.6 ± 1.3	5.6
Sulfate removal	**mg/L**	235 ± 8	264 ± 10	29	329 ± 4	94
	%	56.0 ± 2.0	62.9 ± 2.3	6.9	72.6 ± 0.8	16.6

degraded the hydrocarbon components. Microbial activity in the proximity of electrodes facilitates anaerobic conditions and proceeds for electron transfer between the electrode and TPH degrading bacteria. Moreover, hybrid system that contains both bioanode and biocathode improves the degradation efficiency [35].

The study by Wang et al. [34], also revealed the syntrophic hydrocarbon conversion pathways between aerobic hydrocarbon-degrading bacteria and anodic electroactive bacteria. It was observed that the bioelectrochemical degradation proceeded with electroactive anodic biofilms (EAB) comprising 27 % of *Geobacter*).

Fig. 3. Bioelectrochemical treatment in terms of chemical oxygen demand (COD) through BES under the influence of acetate and sewage in comparison to the control operation (a) Cycle wise performance and (b) Hour wise performance in a single operating cycle.

Aerobic bacteria such as *Parvibaculum* (24 %) and *Pseudomonas* (30 %) were identified in the soil [34]. Considering soil nature in Qatar, the reactor matrix was filled using 70 % of sand along with peat moss. The degradation rates of hydrocarbons in this study also can be attributed to the positive impact of sand [34]. Pilot-scale studies having a volume of 50 L were also carried out using diesel contaminated soils (TPH concentration of 12.25 ± 0.36 g/kg dry soil) that resulted in 89 % TPH_R in 120 days of operation [36]. The high rate of degradation (80 mg/L-day) recorded in the hybrid BES system is attributed to the presence of bioanodic and biocathodic mechanisms and continuous recirculation of PW. Continuous recirculation allows the pollutants to move towards close proximity of the electrodes and facilitated efficient electron transfer.

Substrate degradation occurs due to the bioelectrochemical activity in the soil BES was evaluated by current and power density recorded during respective experimental variations. Among the five operating cycles, bioelectrochemical activity was found to show current in the range of 4.31 mA (917 mW/m^2, Cycle 3) and 4.58 mA (974 mW/m^2, Cycle 4 and 5) (Fig. 4a). On average, 4.5 mA of current and 957 mW/m^2 of power density were registered in this phase (Table 1). The electron transfer pattern in a single batch cycle was correlated well with the substrate (TPH and COD) reduction. At the start of the cycle, 4.21 mA was recorded that gradually improved with time and reached the highest current of 4.4 mA by 72 h of operation (Fig. 4b). Later (72 to 168 h), the system showed current that varies in a narrow range (4.37 and 4.3 mA). In microbial electrolysis systems, increase in electrochemical activity recording is found to be proportional with substrate degradation [37,38]. Similarly, the present study also clearly visualized the function of current generation from the reduction of TPH/COD.
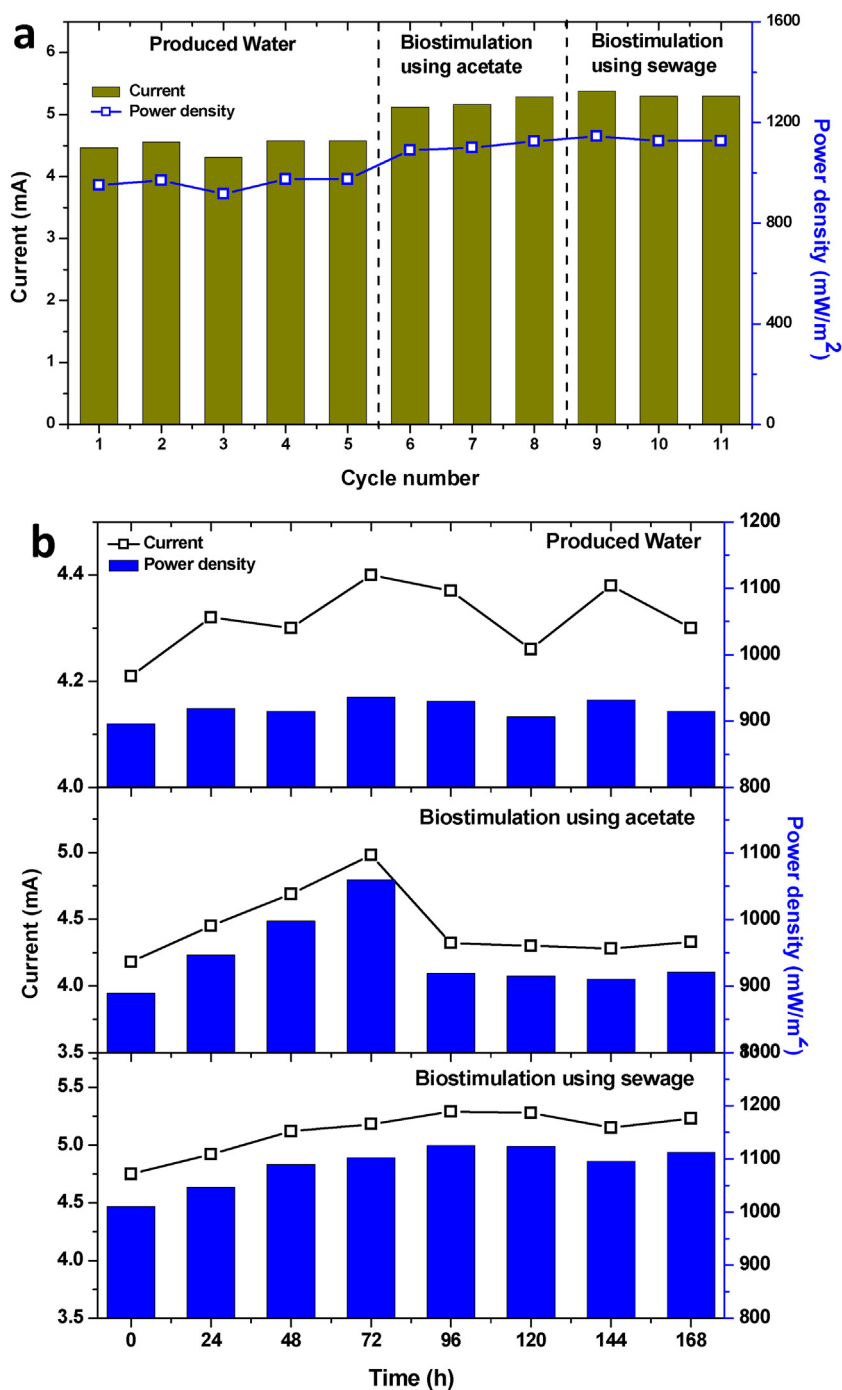
Fig. 4. Bioelectrochemical activity recorded during remediation of petroleum based contaminants in soil with control operation and operation with acetate and sewage (a) Current and power density against 11 cycles of operation (3 phases) (b) Hourly data of current and power density during the 3 phases of operation.

3.2. Influence of acetate as stimulant on bioelectrochemical treatment (BET) of produced water

Bioelectrochemical treatment of PW in soil BES showed about 64.5 ± 4% TPH reduction and 69 ± 2% COD reduction, leaving considerable amounts of pollutants untreated after 7 days of operation. Ingredients of PW are complex and not amenable for bacterial metabolism. Adding simple organics to the system may assist in having more favorable conditions for electrochemically bacterial activity. Acetate is found to be one of the suitable substrates for electrochemically active bacteria in BES. In this direction, 60 mg/L acetate was added to PW to provide favorable

metabolic environment. Acetate in the soil microcosm acts as a potential electron donor that stimulates the indigenous microbial communities, which in turn improves biodegradation [39]. From the three cycles of operation with PW + acetate, a visible improvement in substrate degradation was observed. The maximum TPH_R of 540 mg/L (69.23 %) was recorded during the 6th cycle of operation (Fig. 2a). In the case of COD, the maximum reduction (COD removal, 806 mg/L, 73.27 %) was identified during the 8th cycle of operation (Fig. 3a). Comparing the average reduction values during the second phase (PW + acetate) with the first phase (PW), addition of acetate helped for improvement in removal of TPH (improvement, 22 mg TPH/L) and COD (improvement, 89 mg

COD/L). Hourly analyses of TPH and COD in single cycle operation were also depicted influence of the acetate on substrate removal (Fig. 2b and Fig. 3b). Specific TPH removal rate increased with time until 48 h of operation (TPH$_R$, 75 mg/L-day) and subsequently nearly a stable performance was recorded. In the case of acetate addition to PW, a significant improvement in average TPH removal rate (Phase 1, 69.5 mg/L-day; Phase 2, 73.2 mg/L-day) was recorded. In the case of COD reduction, also acetate addition showed similar influence. The average COD reduction rate of 115.9 mg/L-day (Phase 2), was significantly higher than 102.6 mg/L-day that recorded in Phase 1 (PW). Consolidating soil BES performance with PW as substrate, addition of acetate to PW in minor concentrations resulted in 5.32 % and 12.96 % improvement in TPH and COD removal rates, respectively. Acetate addition stimulated bioelectrochemical activity of soil BES that helped for enhanced substrate degradation.

Addition of organics along with inorganic nutrients such as N and P was found to accelerate the degradation. Addition of waste organics such as poultry droppings, tea leaf, potato skin, cow dung was found to exhibit enhanced TPH removal [40–44]. However, the removal efficiency was found to depend on the type of organic nutrient used for biostimulation [41]. The addition of brewery spent for bioremediation of soil contaminated with spent oil was resulted in 92 % degradation over 84 days of operation, whereas, control operation was shown only 55 % hydrocarbons degradation [41]. The present study executed with acetate addition and operated for 7 days in a bioelectrochemical system resulted in 67.4 % TPH removal efficiency. In another study, biochars that are produced from sawdust and wheat straw also acted as biostimulants for remediation of PAHs-contaminated soil collected from Dagang oil field in Tianjin, China [44]. Biochar addition changed the predominant bacterial community that resulted in extended removal of PAHs in soil. The general process of bioremediation proceeds with oxidation biodegradation and reductive biodegradation mechanisms [45,46]. Along with these natural mechanisms, in the hybrid soil BES system, the presence of bioanode facilitates bioelectrochemical oxidation and biocathodes proceeds with bioelectrochemical reduction reactions. The hybrid configuration of soil BES further improved its degradation efficiency through the addition of acetate as an organic bioelectro-stimulants.

In the case of bioelectrochemical activity, acetate addition also resulted in increased current and power density generation (Fig. 4a). In the three cycles of operation (6th to 8th cycle), the maximum electrochemical activity was identified as 5.29 mA (current) and 1126 mW/m^2 (power density). Compared to PW alone (Phase 1), acetate addition increased the electrochemical activity, demonstrating that acetate stimulated electrochemically active bacteria in the soil electrode vicinity and helped for increased electron transfer. The improvement in the bioelectrochemical activity was accounted for 15.4 %. When the current generation during single cycle of operation observed, interesting findings were recorded with respect to substrate degradation and bioelectrochemical activity. After feeding the soil BES with acetate, gradual increase in current generation was identified up to 72 h (4.98 mA) of operation and later (at 96 h), a substantial drop was observed and a stable current was recorded till the end of the cycle (Fig. 4b). Since acetate is simple and more favorable substrate for bioelectrochemically active bacteria, it was consumed during the first 72 h of the cycle. Later, the metabolism might have shifted to PW constituents, where relatively less current (4.28–4.33 mA) was recorded. Phase 2 operation clearly evidenced positive influence of acetate as stimulant to the bioelectrochemical activity in soil BES. In the case bioelectrochemical systems, also addition of biostimulants exhibited improvement in bioelectrogenesis. In a previous study, supplementation of intact tea-extracts stimulated power generation in microbial fuel cells [47]. Addition of key

nutrients leads to stimulation of the soil microbiome that helps for the degradation of petroleum hydrocarbons [48]. Similarly, in the present study, the inorganic nutrients and biodegradable organic matters present in the raw sewage might have helped to stimulate the bioelectrochemical activity in the vicinity of bioanode and biocathode. In the case of acetate, micronutrients were not provided. This might be acted as controlling factor for limited improvement in bioelectrochemical activity.

3.3. Influence of sewage as stimulant on bioelectrochemical treatment (BET) of produced water

Addition of acetate to PW in soil BES resulted in improvement in TPH$_R$. Even though, residual PW pollutants were found to present in the range of 33 ± 2% and 28 ± 2% for TPH and COD, respectively. For further improvement, raw sewage was added to the PW as supplement with easily biodegradable organics and micronutrients. Raw sewage water provides simple nutrients and different types of organic molecules from biological origin. Supplementing the sewage to PW provided key nutrients for microbial activity and enhanced the degradation in the soil matrix (Figs. 2 and 3). From the operation of the three cycles (9th to 11th cycle), supplementing sewage to PW showed significant improvement in TPH$_R$. An average of 57 mg/L of TPH$_R$ improvement was recorded that accounts for 7.3 % improvement, due to supplementation of sewage (Table 1). Sewage supplementation also showed superior function in improving degradation efficiency than acetate supplementation condition. A maximum TPH removal of 572 mg/L was recorded during the 10th cycle of operation that comprising 73.33 % degradation efficiency (Fig. 2a). Similar to TPH, COD also showed superior function towards treatment due to supplementing of sewage to PW. A maximum COD reduction of 832 mg/L (75.64 %) was also recorded during the 10th cycle of operation (Fig. 3a). Compared to PW as sole substrate in soil BES, sewage supplementation was improved by 117 mg COD/L. Further evaluation of specific TPH removal rate was evidenced influence of sewage addition clearly. The specific TPH degradation rate increased with time and exhibited a maximum by 48 h of operation (84 mg/L) and later it continued as constant until 144 h (Fig. 2b). By 168 h, a drop in TPH$_R$ was recorded (72 mg/L-day). Compared to PW alone (Phase 1) and acetate addition (Phase 2) operations, sewage supplementation showed stable performance for longer period (from 48 to 144 h). Sewage was found to increase stability in the biological activity of anaerobic processes [49,50]. The addition of biostimulants also acts as co-substrate which usually creates positive synergy in anaerobic microenvironment through supplementing the deficient nutrients [50] and also establish proper C/N ratio [51]. The addition of biostimulants also improves the biodegradable organic fraction of the substrate [52,53]. This resulted in increased overall remediation efficiency. The nutrients present in sewage also help to improve the buffering capacity [54]. In the present study, also a stable performance was identified with sewage addition to PW. Previously, several studies were performed using sewage and sewage sludge biostimulation strategy [55–57]. Apart from hydrocarbons degradation, pesticides were also treated by biostimulation using sewage solids [58]. Raw sewage is found to have nitrogen, phosphorus and organics which can accelerate biological activity. In this direction, extending the raw sewage usage to BET of hydrocarbon-contaminated soil showed rapid degradation rate and improved efficiency.

Sewage supplementation also showed visible influence on the electrochemical activity in soil BES (Fig. 4). In all the three cycles of the 3rd phase of operation, current was recorded in the narrow range of 5.3 mA and 5.38 mA (1128–1145 mW/m^2) (Fig. 4a). On an average, 0.83 mA of current and 176 mW/m^2 of power density were improved due to the sewage addition (Table 1). The organics

present in sewage stimulate the bioelectrochemical activity and also help for simultaneous degradation of TPH and other pollutants present in the PW. When the soil BES was evaluated during single cycle of operation, a gradual improvement in current and power density were seen up to 96 h of operation. Later, a relatively slight drop was observed. This phenomenon was found to be different from Phase 1 and 2, suggesting that the organics present in sewage were gradually degraded along with TPH. This helped for longer and extended treatment efficiency of PW.

3.4. Sulfate removal in soil BES during organics supplementation

PW contains high concentration of sulfates. In the present study, PW was found to have 420 mg/L sulfate. This is significantly high that needs to be treated properly in soil matrix. High concentration of sulfates proliferate sulfate reducing bacteria (SRB) growth in the soil matrix [59]. Further, SRB growth causes clogging, and also promotes microbial induced corrosion if any pipeline is placed in the soil. This emphasizes the importance of sulfate removal in PW contaminated soils. BESs were known to exhibit removal of sulfates effectively from petroleum-based wastewater, including PW [60,61]. Theoretically, biological sulfate reduction to sulfide requires eight reducing equivalents. According to which, minimum COD/sulfate of 0.67 (mass ratio) is required to achieve complete removal of sulfates [62–64]. The present study showed effectiveness towards sulfate removal. During Phase 1 (PW only) of operation, a maximum sulfate removal of 58 % (SO_4^{2-} removal, 246 mg/L) was registered during the 3rd cycle (Fig. 5). Addition of acetate to the PW resulted in improvement of sulfate removal (Phase 2, 275 mg/L sulfate removal, 65.5 %). Addition of acetate improves the microbial activity, which further improves the bioelectrochemical activity. This might helped for the enhancement of sulfate removal. According to Pozo et al., [65], cathodic biofilms enhance autotrophic sulfate reduction by sulfate reducing bacteria. Hybrid bioelectrochemical system that contains biocathode helped for enhancement in sulfate removal [30,35]. Bioanodes developed with SRB was found to degrade chlorinated phenol and produce in situ hydrogen peroxide, simultaneously. This helped for the improved bioelectrochemical removal of sulfates and hydrocarbons [62]. In the present study, both bioanodic and biocathodic function for sulfate removal. Further, during evaluation of bioelectro-stimulation using sewage, the net sulfate concentration was increased from 42 mg/L to 453 mg/L due to the sulfate content present in the raw sewage. A significant improvement in sulfate removal was evidenced in Phase 3. A maximum of 333 mg/L of sulfates was removed using sewage as bioelectro-stimulant, which is equivalent to 73.5 % removal. Compared to PW alone as substrate, sulfate removal was increased by 29.1 mg/L
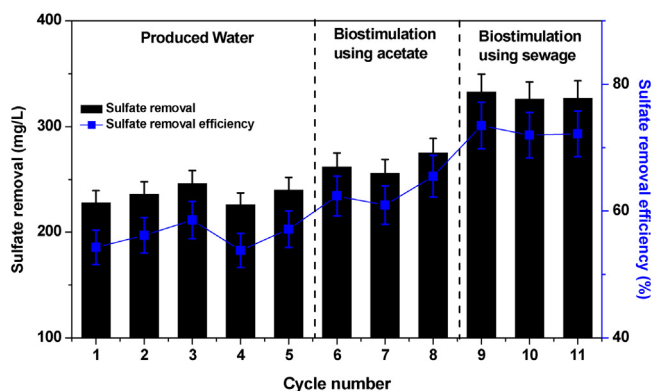

Fig. 5. Removal of sulfates in PW through bioelectrochemical treatment in soil environment under the influence of acetate and sewage in comparison with control operation.
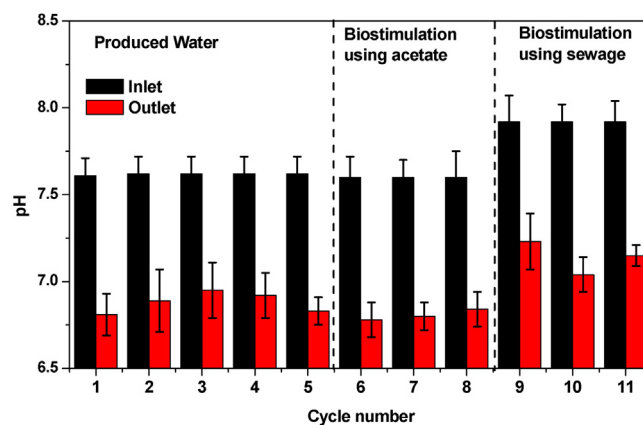

Fig. 6. Change of pH during bioelectrochemical treatment of PW in soil microenvironment under the influence of acetate and sewage as biostimulants in comparison with control operation.

(enhancement, 6.9 %) and 93.5 mg/L (enhancement, 16.6 %) using acetate and sewage as bioelectro-stimulants respectively.

Bioelectro-stimulation was also influenced redox conditions of the soil BES system. pH of the inlet PW was found as 7.61. Soil BES treatment was resulted in a drop of the pH to the range of 6.81–6.95 (Fig. 6). Further, addition of acetate did not affect much the outlet pH that maintained between 6.78 and 6.84. In the case of sewage addition to PW, the outlet pH was sustained in the range of 7.04 and 7.23. Compared to the control operation and acetate as biostimulant, sewage addition showed lower drop in the pH and pH sustained above neutral conditions. Microbiological sulfate removal mechanism is highly influenced by the pH [60]. On the other hand, pH conditions also influence the proliferation of sulfate reducing bacteria. In the present study, change in the pH might influence the sulfate removal.

4. Conclusions

The present study demonstrated the use of sewage as bioelectrochemical activity enhancer for in situ degradation of hydrocarbons in petroleum contaminated soils. The study has simulated PW contamination conditions in the laboratory that showed enhanced reduction function of TPH, COD and sulfates along with bio electrochemical activity. Compared to the control (only PW), an improvement in bioelectrochemical activity was registered as 176 mW/m^2 and 148 mW/m^2, using sewage and acetate as bioelectro-stimulants respectively. Similarly, sewage and acetate also enhanced TPH removal (22 mg/L improvement with acetate; 57 mg/L improvement with sewage) and COD reduction (89 mg/L improvement with acetate; 117 mg/L improvement with sewage). Sewage showed superior and stable effect with respect to bioelectrochemical activity and substrate degradation, whereas acetate showed rapid improvement of bioelectrochemical activity and substrate degradation. This is the first study that shows the effect of bioelectro-stimulation using BES in soil microenvironment for degradation of petroleum hydrocarbons and other contaminants.

CRediT authorship contribution statement

Gunda Mohanakrishna: Conceptualization, Methodology, Formal analysis, Writing - original draft. **Riyadh I. Al-Raoush:** Funding acquisition, Project administration, Resources, Conceptualization, Methodology, Writing - review & editing. **Ibrahim M. Abu-Reesh:** Resources, Conceptualization, Methodology, Writing - review & editing.

Declaration of Competing Interest

There is no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.btre.2020.e00478.

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