Moisture retention materials enhance bioelectrochemical remediation of hydrocarbon-contaminated soil in unsaturated conditions

By

Yixiao Cui

B.S., Missouri University of Science and Technology 2015

A thesis submitted to the

Faculty of the Graduate School of the

University of Colorado in partial fulfillment

Of the requirement for the degree of

Master's of Science

Department of Civil, Architectural, and Environmental Engineering

2017

This thesis entitled:

Moisture retention materials enhance bioelectrochemical remediation of hydrocarbon-contaminated soil in unsaturated conditions

written by Yixiao Cui
has been approved for the Department of Civil, Architectural, and Environmental Engineering

_____

Dr. Zhiyong Jason Ren

_____

Dr. JoAnn Silverstein

_____

Dr. Song Jin

Date_____

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.

Abstract

Cui, Yixiao (Master's, Civil, Architectural, and Environmental Engineering)
Moisture retention material enhanced microbial electrochemical remediation of hydrocarbon-contaminated unsaturated soil
Thesis directed by Associate Professor Zhiyong Jason Ren

Soil contamination has become a growing problem worldwide. Common soil remediation technologies including physical excavation, chemical oxidation or reduction are generally energy and chemical intensive. Compare to them, bioelectrochemical systems (BECSs) is a relatively cost-effective and environmentally friendly way to treat contaminated soil. Much work has been done using BECS on soil remediation, both in laboratory scales and in the field. But many limitations still restrict the application and development of soil BECS. One of the great limitations is the soil moisture level. The contaminants degradation highly relays on the growth of hydrocarbon degradation bacteria (HDB), but the growth of HDB highly correlate with the soil moisture level. The decrease of water content in soil can inhibit bacterial activities and lead to reduced performance of soil BECS. To our best knowledge, few studies investigated the methods to maintain soil BECS under unsaturated soil conditions.

This study aims to find a practical approach to improve soil BECS performance under unsaturated soil conditions. As the bacterial activities mainly occur on the anode of the BECS, water retention layers were added around the anode of BECSs in this study. Hydrogel was selected as the water retention materials in this study because of its excellent water retention capacity. Four reactors were settled in this study: soil and hydrogel mixture water retention layer enhanced BECS reactor (SHB), graphite granule and hydrogel mixture layer enhanced BECS reactor (GHB), soil and BECS reactor (SB), and a control tank without BECS (Con). During the 110 days of study, all three BECSs showed great

improvement on total petroleum hydrocarbon (TPH) degradation compare to the control. Among the three BECSs, SHB showed the best current production and TPH degradation. During each evaporation test that have been proceed, SHB maintained system performance 40% longer than the SB in unsaturated condition, and this extension increased to 90% compare to GHB. SHB also had the highest TPH removal (37% TPH removal by day 110) among the three BECSs, which is 42% higher than SB, 164% higher than GHB, and 517% higher than control. These result shows the feasibility to use hydrogel as a water retention material to enhance soil BECS performance under unsaturated soil conditions.

Dedication

This thesis is dedicated first to my parents Hanyun Cui and Sufang Yao for their support in my endeavors through all these years that have led me to where I am today. To all of my family, friends, and loved ones who have always been there to support me, congratulate me, and shared memories in my life.

Dedication

Acknowledgments

I must acknowledge my advisor Dr. Zhiyong "Jason" Ren for his guidance throughout my first research project. His support has been essential in my development as a researcher and science engineer. To my committee members, Prof. Joann Silverstein and Dr. Jin, thank you for taking time to review my thesis and provide valuable feedback. To my colleagues, Lu Lu and Zheng Ge, thank you for all your valuable advices on this research project. To the Ren lab team, thank you for making my time at the University of Colorado wonderful and meaningful. I must also acknowledge the Chevron Company for their support of my projects through funding.

Contents

List of Tables

List of Figures

Introduction

Soil pollution keeps increasing in the last century with the bloom of industrial activities. In the past century, the production of oil increased fast due to the huge demand of petroleum, which enlarged the petroleum hydrocarbon polluted area. Petroleum leaks or spills can result in elevated levels of contaminants include benzene, toluene, and xylene in the soil. During the last decade, the United States Environmental Protection Agency (US EPA) had spent $243 million per year for highly contaminated Superfund site cleanup, and this number expected to be increase in the next decade (EPA, 2011). Physical excavation, chemical oxidation or reduction, and bioremediation are common technologies have been used for soil remediation. As physical excavation and chemical oxidation/reduction are generally energy and chemical intensive, bioremediation is considered as a cost-effective and environmentally friendly way to treat contaminated soil. However, the performance of bioremediation is primarily governed by the following challenges: 1. lack of contact between the contaminants and amendments due to the heterogeneous matrix, 2. the limitation of the electron donor/acceptor availability, 3. slow kinetics because the above issues (Li, Wang et al. 2015).

Due to the limitations of bioremediation discussed above, a new energy positive bioremediation process called bioelectrochemical systems (BECSs) has been introduced for enhanced and accelerated remediation. Traditional BES reactors consist of the anode, the cathode and an optional separator. Electron donor type of substrates can be oxidized by microorganisms in the anode chamber to generate electron flow to the cathode located in the cathode chamber. The electrons (current) can be used for direct electricity production, or used to reduce electron acceptor type of chemicals and produce less toxic or value-added products (Li, Wang et al. 2015). Microbial fuel cells (MFCs) is an example of BES that has

direct electricity production. Electrochemically active bacteria (EAB) serve on the anode of an MFC, catalyze the oxidization of organic electron donors such as hydrocarbon, and transfer electrons to the anode extracellularly. Electrons are then transferred through an external circuit to the cathode, where typically O_2 is reduced to H_2O. The current produced by MFC can power wireless sensors for remote online monitoring (Lu, Huggins et al. 2014).

The BES enhanced hydrocarbon remediation has many unique advantages. During the process, the anode serves as an intermediate and non-exhaustible electron acceptor, transferring electrons from the contaminants to an abundant source of terminal electron acceptors such as O_2 in the air (Lu, Huggins et al. 2014). Therefore, the entire process does not require any chemical addition or energy input, and even generates energy during the process. The energy can be harvested with the voltage boosted to required levels for usage. While most of other processes can only provide one redox condition, BESs can provide oxidation reactions on the anode and reduction reaction on the cathode. This integrated microbial-electrochemical removal mechanism can remove complex contaminants with different characteristics. Petroleum hydrocarbon and biofuels can be used as electron donors and oxidized using the anode. Metal ions, perchlorate, and chlorinated solvents, etc. can be used as electron acceptors and reduced using the cathode (Li, Wang et al. 2015).

Many studies have been done in soil BES field, but almost all of them used saturated soil conditions in their studies (Morris and Jin 2012, Lu, Huggins et al. 2014, Lu, Yazdi et al. 2014, Li, Wang et al. 2015, Mao, Lu et al. 2016). Because dry soil can cause various problems and lead to the failure of BECS system. The study of Wang et al.,2012 shows that the decrease of water content in soil MFC can limit the growth of hydrocarbon degradation bacteria (HDB), inhibit the proton diffusion and bacterial activity. In their study, the total charge generated from soil MFC decreased from 125 ±7 C to 15 ±2 C when the water content of soil decreased from 33% to 23%. The thermodynamic properties dictate that the internal water

potential of organisms is the same with the water potential of the external soil solution which they inhabit. Passive equilibration of internal water potential can occur when the water potential of the external soil solution changes. But such passive response has limit utility in maintaining physiological activity and surviving the stress of soil drying. When a soil becomes too dry for the organisms, passive equilibration may lead to cell death (Kieft, soroker et al. 1987). Thus, the electrochemically active bacteria cannot survive under dry soil conditions. Unfortunately, many contaminated fields are not saturated at most of the time. The groundwater level fluctuates over seasons, so holding moisture during dry seasons is needed to keep BECS performance. As electrochemically active bacteria live on the anode of BECS, keep the humidity of the anode may improve the survival of bacteria under unsaturated soil condition. One of the most advantages of BES application for soil remediation is that it is an in-situ application. Therefore, applying a water retention layer around the anode is a feasible and effective way to improve the anode humidity. So far, no study looked at the application of water retention layers around the anode. The water retention materials that is applied to the anode need to be environment friendly, non-toxic, long lasting, no negative effects toward BECS system, and cost effective. This study compared several water retention materials in the market, and tested using polyacrylamide hydrogel as the water retention material to improve the soil MFC performance under unsaturated soil conditions.

Methods and Materials

Soil Sample Collection and Characterization

Soil samples were collected from Santa Rosa, CA, USA. The sampling location has been contaminated by petroleum hydrocarbons. Soil samples were

partially air-dried, and sieved to <2 mm to remove rocks and plant roots. Sieved soil samples were stored at 4 °C before use. The main characteristics of the soil samples are summarized in Table 1. The soil sample was characterized as clay loam according to USDA soil texture classification. The soil samples were mixed with play sand (Home Depot) in a 1:1 ratio to improve mass transfer. After mixing, soil was characterized as sandy soil. The sandy soil was well mixed with diesel to reach the TPH concentration of 11.34 ± 3.26 g/kg-dry soil. Soils were saturated with tap water. Saturated diesel contaminated sandy soil were applied to the reactors. The characteristics of soil initially been applied to the reactors are shown in Table 2. No nutrient was added to the soil during the first two week of running. During the operation, artificial groundwater was added to each reactor to remain

Table 1. Characteristics of the raw soil samples

Parameter	Value
pH	6.59 ± 0.05
Conductivity (µS/cm)	0.10 ± 0.01
% sand (2.0-0.05 mm)	40.7%
% silt (0.05-0.002 mm)	26.0%
% clay (0.002- mm)	33.3%
Total organic carbon (g/kg-DS)	1.5 ± 0.1
Dissolved organic carbon (g/kg-DS)	0.06 ± 0.01
Total petroleum hydrocarbons (g/kg-DS)	0.39 ± 0.08
Nitrate (mg/kg-DS)	0.49 ± 0.01
Phosphate (mg/kg-DS)	<1.5
Sulfate (mg/kg-DS)	<40
Ammonia nitrogen (mg/kg-DS)	<1

Table 2. Characteristics of the initial soil applied to reactors

Parameter	Value
pH	7.27 ± 0.08
Conductivity (mS/cm)	0.61 ± 0.01
% sand (2.0-0.05 mm)	69.4%
% silt (0.05-0.002 mm)	20.0%
% clay (0.002- mm)	10.6%
Total organic carbon (g/kg-DS)	28.5 ± 2.56
Dissolved organic carbon (g/kg-DS)	0.14 ± 0.04
Total petroleum hydrocarbons (g/kg-DS)	11.34 ± 3.26
Nitrate (mg/kg-DS)	1.90 ± 0.07
Phosphate (mg/kg-DS)	1.91 ± 0.03
Sulfate (mg/kg-DS)	<40
Ammonia nitrogen (mg/kg-DS)	<1

soil saturated. Artificial groundwater refers to a solution of 4 mM Na^+, 0.2 mM Ca^{2+}, 0.1 mM Mg^{2+}, 2.4 mM Cl^-, 2 mM HCO_3^-, and 0.1 mM SO_4^{2-} in deionized water (conductivity $\approx$ 0.05 S/m at 22 ° C, pH $\approx$ 8.2) (Mao, Lu et al. 2016). After been running for 15 days, nitrogen (Urea, N-47%) and phosphorus (Tri Super phosphate, TSP, P-45%) were added to soil to improve nutrient to the Total N concentration of 500 mg/kg-DS and available phosphorus concentration of 250 mg/kg-DS.

Water retention materials selection

Among the many water retention materials in the market, carbon felt (Mersen Inc.), water retention foam sheet (Misumi, SOFRAS), and polyacrylamide hydrogel (Water Storing Crystals, Home Depot) are the ones best conform to the above requirements. Water retention test have been done based on three materials.

Three materials with the same volume and same evaporation area were set under room temperature for evaporation. The results of water retention test are shown in Figure 1. The results show that polyacrylamide hydrogel has the best water retention capacity among the three materials been tested. Compare to the other two materials, polyacrylamide hydrogel is the only one that is actually designed to apply in the soil. Polyacrylamide hydrogel is a non-toxic water absorbing polymer that has been widely used in gardening to helps prevent over and under watering. The polyacrylamide hydrogel crystals selling in the market are dry hydrogel crystals that swell when absorb water. The crystals can absorb over one hundred times its own weight of water. After hydrogel become saturated, it storage water in its self when the surrounding environment is also saturated, and release water when the surrounding environment become dry. By mixing polyacrylamide hydrogel with soil can prevent soil from over dry. This study use polyacrylamide hydrogel as the water retention material to improve the MFC performance under unsaturated soil conditions.
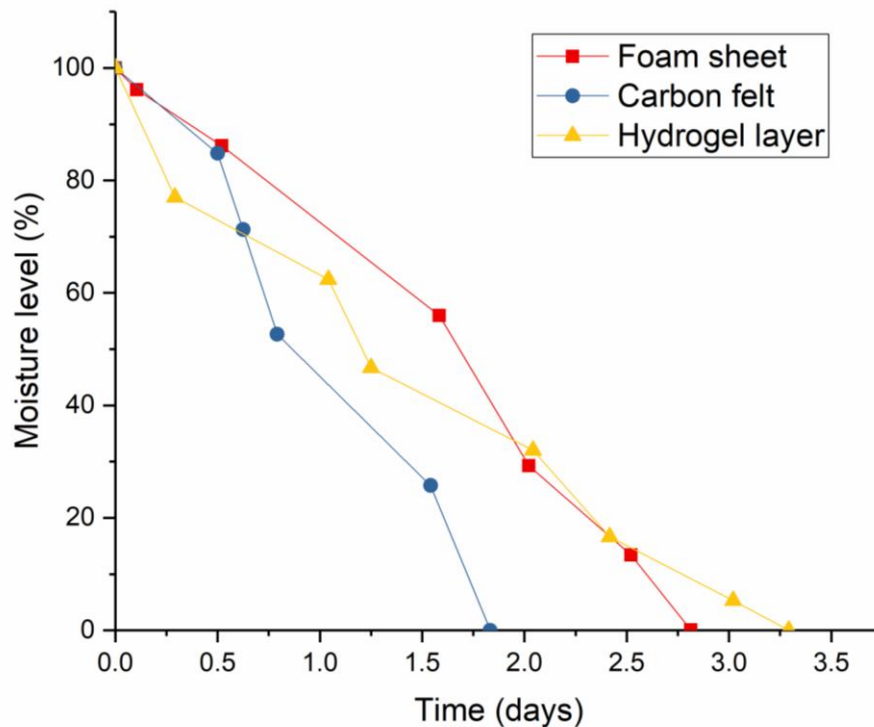

Figure 1. Water retention test of selected materials

The tubular BECSs were inserted into tanks with dimensions of 40 cm × 6 cm × 20 cm (Figure 2) containing saturated diesel-contaminated soil. The BECS reactor was placed on the location shown in Figure 2. Four reactors were designed in this study: a graphite granule and hydrogel enhanced BECS reactor (GHB), a graphite granule enhanced BECS reactor (GGB), a soil and BECS reactor (SB), and a control tank without BECS (Con). Graphite granule was introduced to this study to simulate the condition of pilot BECS reactors on field. Graphite granule was used on the field to fill the gap between BECS reactors and soil. The same graphite granule (diameter <1mm) was used in this study. In GGB, a graphite granule layer of 2 cm was filled in surrounding the BECS reactor. In GHB, graphite granule was mixed with hydrogel in 2:1 ratio by volume, and surround the BES reactor in a layer of 2 cm. The comparison of different graphite granule
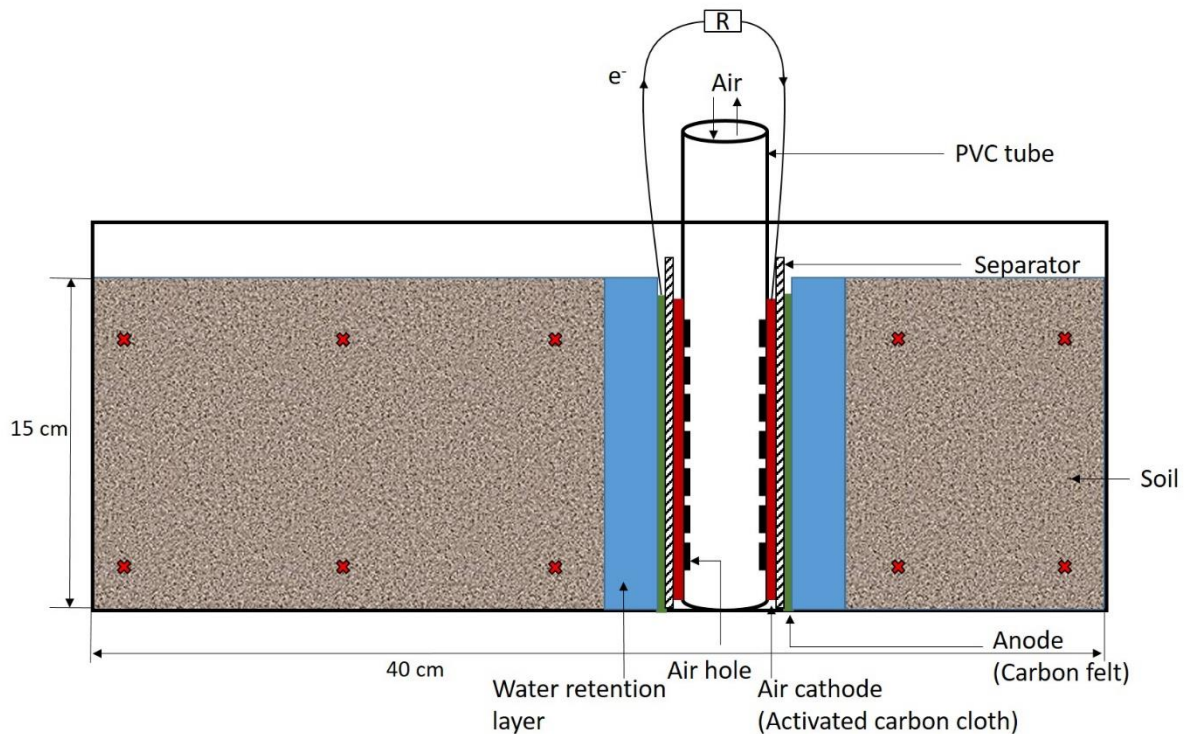

Figure 2. Schematic of tubular BECS design. Sampling locations are marked as red points.

to hydrogel ratios is provided in Appendix B. The SB did not have any layer surround. After been running for 22 days, the graphite granule shows negative impact on BECS, so the graphite granule in GB reactor was removed. Instead of using graphite granule layer, soil and hydrogel mixture (2:1 ratio by volume) was added around the BECS in a layer of 2 cm. After day 22, GGB was replaced by soil and hydrogel enhanced BECS reactor (SHB).

Soil Tubular BECS Construction and Operation

Tubular BECSs were used in this study. In these BECSs reactors, carbon cloth (11 cm × 12 cm, Chemviron Carbon Ltd., PA) with four PTFE diffusion layers (Wang et al., 2011) and activated carbon catalyst served as the air cathode, and carbon felt (14 cm × 13 cm, Mersen Inc., MI) served as the anode, nonconductive permeable J-cloth (4 layers) was used as separator between the cathode and the anode. Each reactor was wrapped around a perforated PVC tube (L 25 cm × D 3.5 cm) with the cathode layer facing inside and exposed to air, and the anode facing outside exposed to soil (Figure 2). A 150 Ω external resistor was connected between the anode and the cathode. Continuous current generated from each reactor was monitored and evaluated by the external circuit with 150 Ω external resistor.

During the operation, soil in all the reactors were covered to minimize evaporation. Artificial groundwater was added to each reactor twice a week to maintain saturated condition. Three evaporation tests were proceeding for each reactor. For each evaporation test, all reactors were uncovered for evaporation, changes on current levels were recorded. After each evaporation test, each reactor was re-saturated by artificial groundwater and covered.

Soil Sampling and Analysis

Soil samples were taken at three distances (2 cm, 10 cm, and 20 cm) away

from the reactor (Figure 2) for TPH measurements. At each location, soil from both top and bottom of the tank were sampled. 3 g of soil were taking from each sampling point and well mixed for TPH measurements. TPH of soil samples were analyzed using modified EPA Method 8015D (USEPA, 2003) and Massachusetts EPH (Massachusetts EPH, 2004). For each soil sample, 3 g of the soil was blended with anhydrous Na2 SO4 until the soil flows freely, then the mixture was mixed with 15 mL of dichloromethane (DCM) followed by extraction using vortex with maximum speed for 5 min. The suspension was centrifuged at 3,000 RPM for 5 min, and the supernatant was filtered through a PTFE 0.22μm filter. This procedure was repeated 3 times, and the combined extractants were concentrated to 2 mL using a nitrogen evaporator. Then 0.8 mL of concentrate was transferred to a GC glass vial for analysis. TPH was measured using a Thermo TRACE GC Ultra Gas Chromatograph equipped with a Restek Rxi-1ms column (20 m × 0.18 mm ID, 0.18 μ m film thickness), split injector, and flame ionization detector. More details of TPH measurement process is described in Appendix A.

Five TPH measurements were conducted at day 0, 25, 50, 80, and 110 during the whole experiment. All soil analytical results were reported on a dry-weight basis. Soil conductivity and pH were measured at the beginning and the end of the experiment, in a 1:5 (w/v) soil: deionized water mixture. Soil particle size distribution was evaluated by the method described in Kettler et al. (2001). The soil total organic carbon (STOC) was analysis by Huffman Hazen Laboratories (Golden, CO). Available nitrate, ammonia nitrogen, phosphate, and sulfate in soil were extracted according to Mussa et al. (2009), and tested using HACH TNT.

Voltage across the external resistor (R=150 Ω) of each reactor was recorded every 10 minutes by using a data acquisition system (model 2700, Keithley Inc.) Current was determined by voltage and external resistor of each reactor. Current density (J, mA/m^2) and power density (P, mW/ m^2) were calculated as $J = U/RA$ and $P = JU$, where A is the effective cathode area ($A = 0.007$ m^2).

Results and Discussion

Soil moisture level effect on current

Before the nutrient was added to the reactor (day 16), no significant current increases occur in reactors. After the nutrient was added to the reactor at day 16, the current in SB started to increase gradually. While the current in GGB and GHB did not have any increase. This reflect that graphite granule may have negative impact towards BECS. After seven days observation, still no improvement occurred in GGB and GHB. GH was replaced by SHB since day 22, while GHB was kept for continue monitoring. The current of SHB stared to increase immediately and continually from day 22. The current of GHB was not increase till day 43.

The current of SB reached its peak at day 38, then started to decrease gradually. The maximum current density for SB was 181.5 ± 0.1 mA/m2 with the corresponding voltages (across 150 Ω resistant) of 353.9 ± 0.1 mV. At day 52, the current of SHB got stable, and remain stabled at the maximum level for about 18 days before started to drop. SHB reached its maximum current density (187.7 ± 0.1 mA/m^2) at day 67, which was higher than SB. The current of GHB getting stabled from day 65, but not as stable as SB and SHB. GHB reached its peak at day 80, its maximum current density was 171.1 ± 0.1 mA/m^2 with the corresponding voltages of 333.7 ± 0.1 mV, which is the lowest maximum current density among the three BECS reactors.
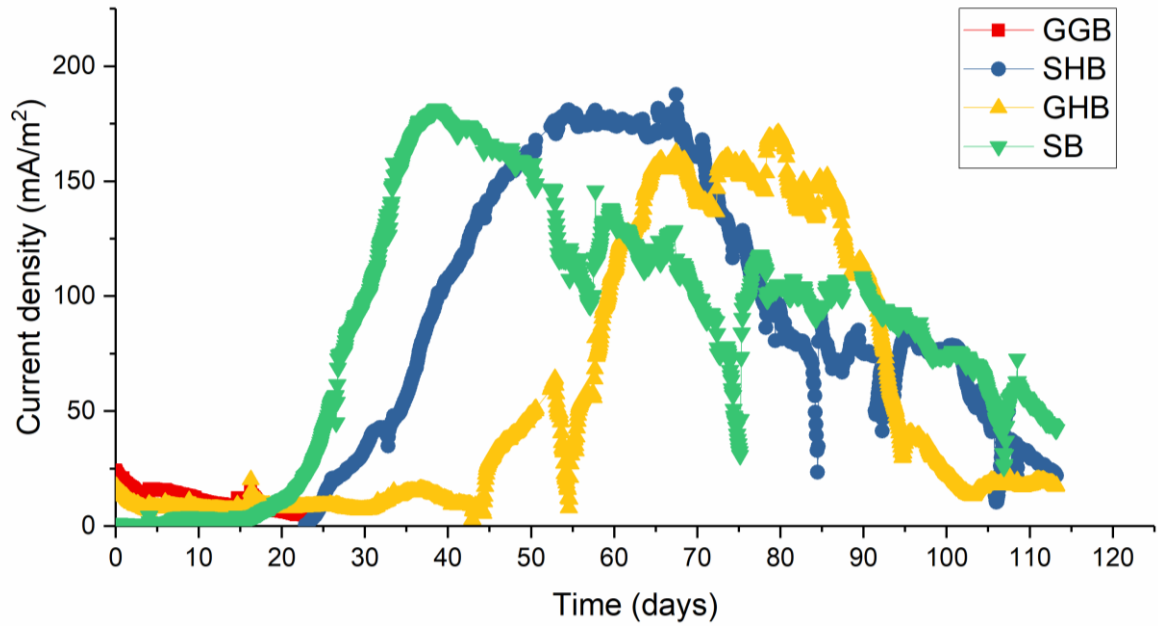
Figure 3. The current density of graphite granule and hydrogel enhanced BECS reactor (GHB), graphite granule enhanced BECS reactor (GGB), soil and BECS reactor (SB), and soil and hydrogel enhanced BECS reactor (SHB).

Three evaporation tests were proceeding during the 110 days running. All four reactors were saturated and covered before each evaporation test, and were uncovered for evaporation during the test. After each test, each reactor was re-saturated by artificial groundwater and covered again.

The first evaporation test was proceeding in the fume hood at day 52, all four reactors were moved to fume hood and uncovered for about 5 days. During the 5 days of evaporation, the anode of each BECS were moistened at the third day, all reactors were saturated on day 4, and finally, all reactors were saturated and covered on day 6. The current density changes of the three BECS reactors are shown in Figure 4. The current density of SB and GHB start dropping significantly after the evaporation started. The current density of SB dropped from 140.5 mA/m² to 115.8 mA/m² in the first half day of evaporation, then remain stabled at 116 mA/m² ± 2 mA/m² in the next one and half days. The current density of GHB dropped continually from 64.2 mA/m² to 8.0 mA/m² in the first 2 days of
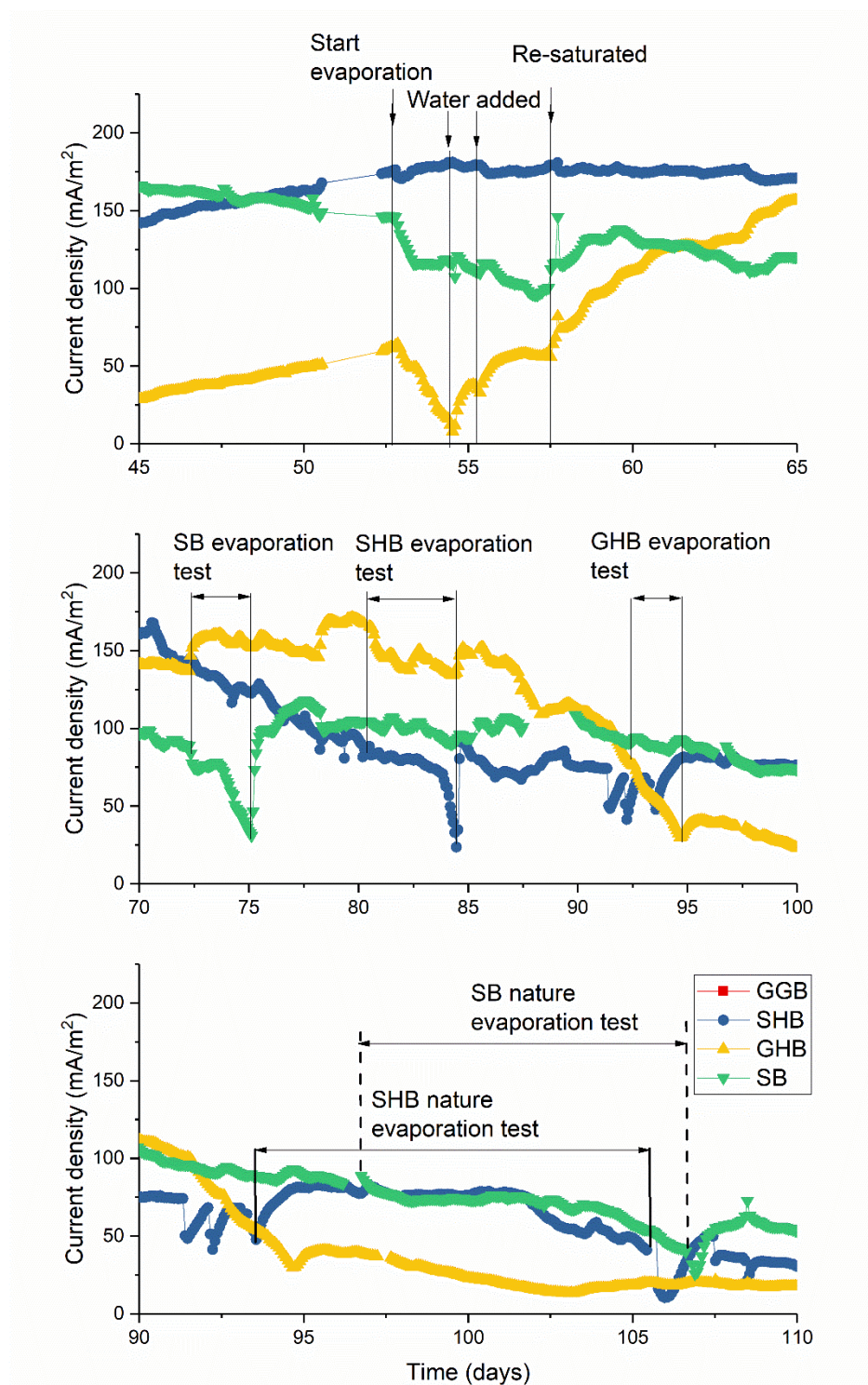
11

Figure 4. Current change during the three evaporation tests. Top: fume hood evaporation test one. Middle: fume hood evaporation test two. Bottom: nature evaporation test.

evaporation. After the anode of each BECS was moistened, the current density of GHB increased immediately from 8.0 mA/m² to 38.1 mA/m² in half day, the current

density of SB increased from 116.0 mA/m² to 120.8 mA/m². After been saturated on day 4, current density of GHB increased from 32.8 mA/m² to 58.2 mA/m² in one day, the current density of SB increased from 110.8 mA/m² to 116.3 mA/m². After been re-saturated and covered, the current density of SB and GHB started to increase gradually and continually. The current density of SHB did not has any impact during the 5-days evaporation test, its current density increased from 171.5 mA/m² to 181.1 mA/m² in these 5 days. In this fume hood evaporation test, SHB showed great performance stability under dry environment compare to SB and GHB. The drastic continually current drop of GHB showed that graphite gradual has poor water retention capacity compare to soil. The SB had a drastic current drop in the first half day of evaporation, then remain a slowly current decrease until been recovered. These may have caused by the sudden change of the environment. High air flow in fume hood may cause the increase of oxygen level in soil and the anode, so impact the performance of the anode, and caused the current drop in SB. By adding hydrogel in the soil around the anode can inhibit the increase of oxygen level in the anode, so ensured the BECS performance in SHB during the fume hood evaporation test.

The second fume hood evaporation proceeded when each reactor's current density was at about 85 ± 5 mA/m², and stopped when the current density decreased to 40 ± 5 mA/m². The current density changes of the three BECS reactors are shown in Figure 4. The current density of SB dropped immediately from 88.5 mA/m² to 73.9 mA/m² in the first half day of evaporation, and then remain stabled at 76 ± 2 mA/m² for one day before decreasing again, which was the similar appearance with the first fume hood evaporation test. Then the current density dropped continually to 40.1 mA/m² in one day. The evaporation test for SB last for 2.8 days, and the current density dropped 48.4 mA/m². In the second fume hood evaporation test of SHB, the current density remain stabled at 84 ± 4 mA/m² for the first 2 days, then slowly decreased from to 70.8 mA/m² in the next one and half days, then rapidly decreased to 39.6 mA/m² in the last half day. The

evaporation test for SHB last for 4 days, and the current density dropped 48.9 mA/m². For GHB, the current started to drop immediately and continually during the second fume hood evaporation, which was similar with the first time. The evaporation test for GHB last for 2 days, and the current density dropped 48.9 mA/m². Soil moisture level of each reactor by the end of the second fume hood evaporation test is shown in Table 3. Soil moisture level were tested using soil moisture meter, which present the soil moisture in 10 levels. Level 10 present as fully saturated, level 0 as dry soil. The soil moisture levels were tested at 15 cm away from each BECS to avoid disturb toward the anode. By the end of evaporation test, the current drop of three reactors were the same, but GHB has the highest soil moisture level, and SHB has the lowest. This means that hydrogel layer has the best water retention capacity, which can keep the anode moist under dry environment, so delayed the current drop in SHB. Graphite granule has the worst water retention capacity, even the soil still has moisture, the graphite granule layer around the anode already dries up. The current change during the evaporation test also showed that SHB has the best performance stability under dry environment compare to SB and GHB. SHB delayed the time of current drop by 42.8% compare to SB, and 90% compare to GHB. After been re-saturated, the current of SB and SHB increased immediately to the current level before the evaporation test, but the current of GHB only increased 0.14 mA, then started to decrease again. The potential test of GHB shows that its anode potential was 34 mV, its cathode potential was 135 mV. The evaporation test may have damaged the anode in GHB because of the poor water retention capacity of graphite granule.

Table 3. Soil moisture level by the end of the second fume hood evaporation test

	Top	Medium	Bottom
SB	4	7	10
SHB	3	7	10
GHB	6	8	10

The third evaporation test was proceeding in room temperature next to the window to mimic the evaporation in nature environment. The current density changes of the three BECS reactors are shown in Figure 4. The current density of SHB remain stabled for 9 days after the evaporation test began, then started to drop slowly. The nature evaporation of SHB last for 13 days, and the current density dropped 41.7 mA/m². For SB, its current density remain stabled for 6 days, then started to drop slowly. The nature evaporation of SB last for 9 days, and the current density dropped 41.2 mA/m². SHB delayed the time of current drop by 44.4% compare to SB. The current of GHB keep dropping after the second evaporation test, so was not able to proceed for nature evaporation test. Soil moisture levels of SB and SHB by the end of the evaporation test are shown in Table 4. Similar with the fume hood evaporation test, SHB last longer during the test, so the soil was dryer than SB, but hydrogel improved the water retention capacity around the anode, so delayed the current drop in SHB.

Table 4. Soil moisture level by the end of the nature evaporation test

	Top	Medium	Bottom
SB	5	8	10
SHB	4	7	10

For all the three evaporation tests, SHB showed the best stability among the three reactors. The result proved that by mixing hydrogel in soil around the anode of BECS can provide moisture to the anode under dry soil conditions, and improve the BECS performance under dry environment.

Petroleum hydrocarbon degradation

The TPH degradation at different distances (2, 10, 20 cm) from each BECS reactor was shown in Figure 5. The soil samples taken from top and bottom of each

distance were analyzed on day 25, 50, 80, and day 110. All three BECS showed

much higher TPH removals compare to the control, in which only 6% TPH was
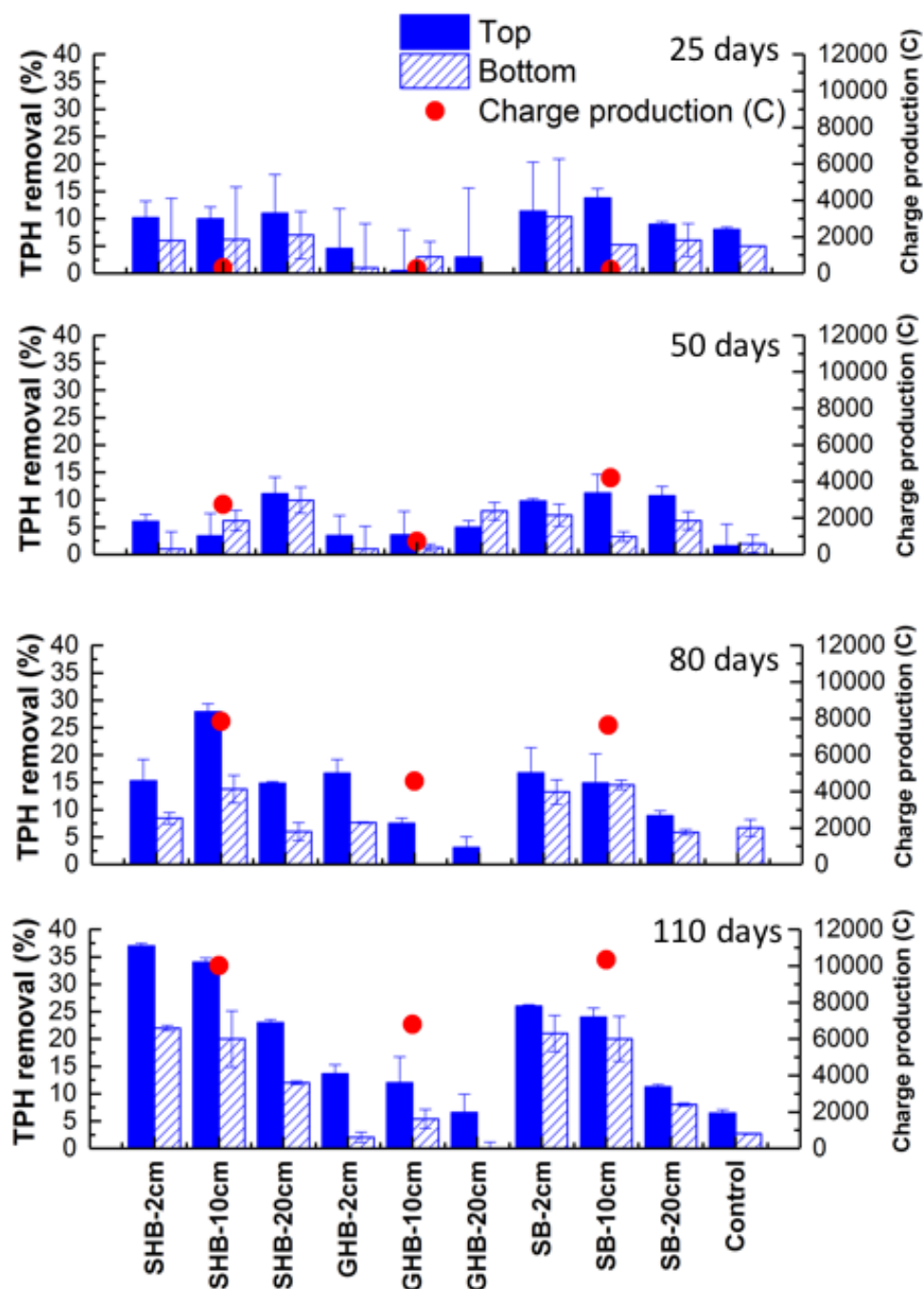

Figure 5. Total petroleum hydrocarbon (TPH) removal from soil and hydrogel enhanced BECS reactor (SHB), graphite granule and hydrogel enhanced BECS reactor (GHB), soil and BECS reactor (SB), and control tank without BECS (Control) at three distances from the anode (2 cm, 10 cm, and 20 cm). Red scatters show the charge production of each BECS at the time of each sampling.

removed. Soil samples taken from top of each reactor shows higher TPH removal than the soil samples from the bottom. This may due to the exposure of oxygen of the surface soil, which give better degradation on the top portion soil. Among the three reactors with BECS, SHB had the highest TPH removal after 110 days running, while GHB had the lowest TPH removal. Up to day 110, SHB has removed 37% TPH at 2cm, 34% TPH at 10cm, and 23% TPH at 20cm. SHB at 2 cm had 517% increase in degradation efficiency compare to control, and 42% increase compare to SB at 2 cm, in which 26% TPH was removed. GHB has the lowest TPH removal because it had the lowest charge production among the three BECS reactors. The total charge generated of each BECS by the time of each sampling are shown in Figure 5. The total charge generated is presented using total Coulombic output (Q, C), which was obtained by $Q = \int_0^T (E/R)dt$, where T (s) is the cycle time. The TPH removal have a positive correlation with charge productions. Both SHB and SB have over 10000 C charge production after 110 days running, while GHB only has about 7000 C. The charge production of GHB was the lowest among three BECSs at each sampling, and so does its TPH removal. By day 110, each reactor has the highest TPH removal at the 2 cm from the anode, the TPH degradation decline as the distance extended from the anodes. Compare to 2 cm distance, TPH degradation declined 7.0%-14.3% at 10 cm, and 37.8%-57.7% at 20 cm.

Biodegradation of n-alkanes

To study the degradation of hydrocarbons in soil, n-alkanes were analyzed for soil samples at 2 cm from the anode of each BECS. N-alkanes are the main constituents of diesel, alkanes containing C8 to C30 were detected in soil samples. The heat map of the removal of n-alkanes with different carbon numbers is shown in Figure 6. The blue color presents lower concentrations of the n-alkanes, and red color presents higher concentrations. The heat map shows similar result with TPH

removals, all three BECS reactors have much higher n-alkanes removal than the control, SHB and SB have better removals compare to GHB. The heat map also shows higher removal toward lower carbon number n-alkanes. Nearly all n-alkanes with 8-10 carbons in the three BECS reactors were degraded in 80 days.
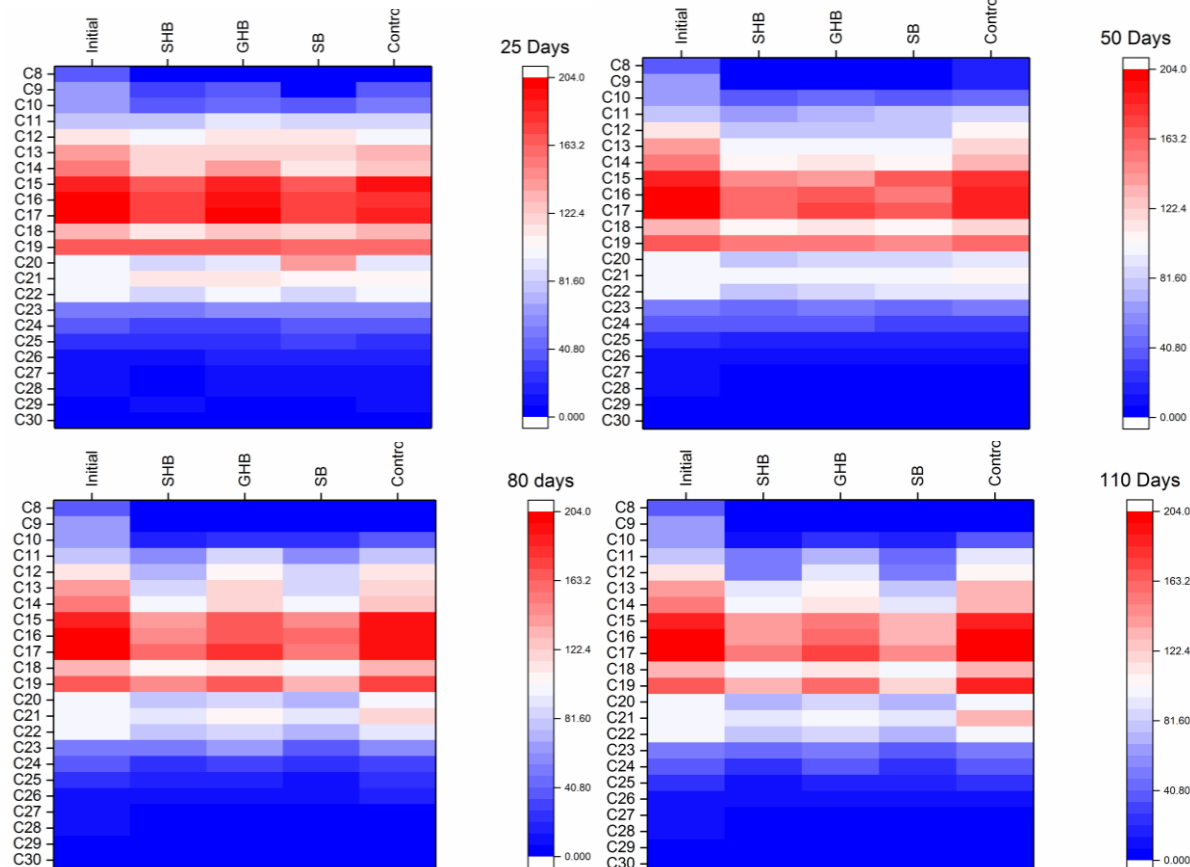

Figure 6. The concentration changes of n-alkanes in reactors at each sampling times. The horizontal axis indicates the soil samples from different reactors (2 cm from the anode of each BECS), the vertical axis indicates the removal of a-alkanes with different carbon numbers.

By the end of the study, the concentration of C8-C10 portion degraded from 163 mg/kg-DS to an average of 28 mg/kg-DS, which is an 83% removal.

Soil pH and electrical conductivity changes

The pH and conductivity change through the 110 days in all four reactors are shown in Figure 7. The soil pH increased continually in 110 days. The increase of soil pH might due to two reasons: the alkalinity of artificial groundwater, and

the alkalinity of sand. Soil was saturated with tap water (pH = 7.15) at day 0, artificial groundwater with pH around 8.4 was added to each reactor to remain soil saturated during the rest operations. Because of evaporation tests, liters of artificial ground water have been added into each reactor during the 110 days running, and this caused the increase of soil pH. The other import factor is the
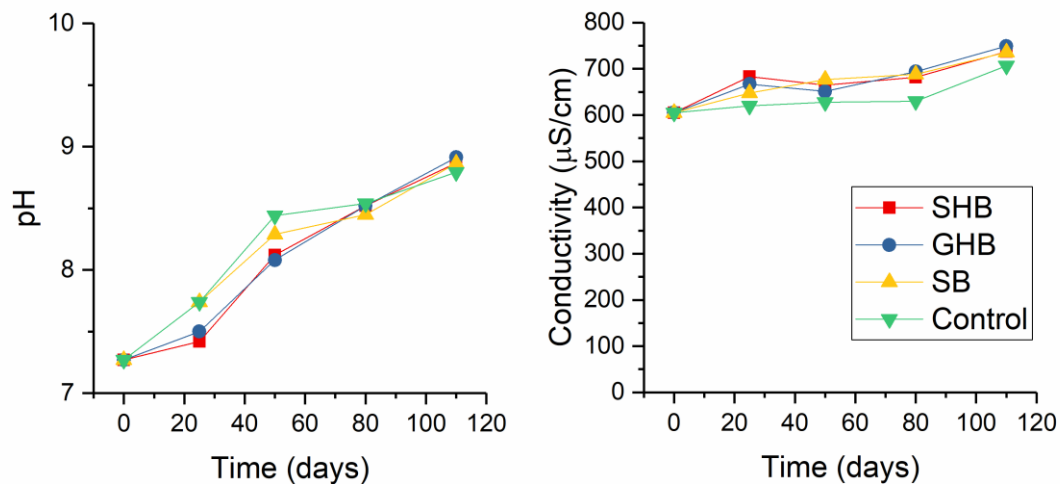

Figure 7. pH and conductivity change in each reactor

alkalinity of sand. Play sand were mixed with soil in 1:1 (w:w) ratio. Play sand has pH of 11.07, the mixture of play sand and soil has pH of 9.46, the mixture soil with diesel added (initial soil sample) has pH of 7.27. Diesel may cover the surface of sand and soil particles, inhibit ions from dissolving into water. After diesel been dissolved in water and degraded by bacteria, more ions were released and may cause the increase of pH. In the first 50 days, the increase of pH more due to artificial groundwater; in the last 60 days, pH increased slower compare to the first 50 days, which was due to the release of ions from sand and soil.

The electrical conductivity (EC) increased from 605 μS/cm to about 730 μS/cm in 110 days. The increase of EC was caused by the introduce of artificial groundwater and the ions releasing from soil and sand. The generation of organic acids near the anodes may cause the dissolution of minerals and release ions, also increase the ionic strength in the reactor (Lu, et al., 2014).

Conclusion

The soil and hydrogel mixture water retention layer enhanced BECS reactor (SHB) showed improvement on both current production and TPH degradation compare to the original BECS reactor (SB) in this study. During each evaporation test that have been proceed, SHB delayed the current drop by more than 40% compare to SB. The graphite granule and hydrogel mixture layer enhanced BECS (GHB) shows the least current production and TPH degradation. Graphite granule showed poor water retention capacity in this study, and had negative impact on the anode. TPH degradation showed a strong positive correlation with charge production. Both SHB and SB had over 10000 C charge production after 110 days running, while GHB only had less than 7000 C. GHB also showed the lowest TPH removal among the three BECSs (14% TPH removal at 2 cm location by day 110). SHB had the highest TPH removal (37% TPH removal at 2 cm location by day 110) among the three BECSs, which is 42% higher than SB, 164% higher than GHB, and 517% higher than the control. These result shows the feasibility to use hydrogel as the water retention material to enhance BECS performance under unsaturated soil conditions. By increase the fraction of hydrogel in soil or thickening the water retention layer around the anode may farther improve the performance.

References

Kettler, T. A.; Doran, J. W.; Gilbert, T. L. Simplified method for soil particle-size determination to accompany soil-quality analyses. Soil Sci. Soc. Am. J. 2001, 65 (3), 849−852

Kieft, T. L., et al. (1987). "Microbial biomass response to a rapid increase in water potential when dry soil is wetted." Soil Biology and Biochemistry **19**(2): 119-126.

Li, X., et al. (2015). "Sand amendment enhances bioelectrochemical remediation of petroleum hydrocarbon contaminated soil." Chemosphere **141**: 62-70.

Liu, X., et al. (2013). "Improved microbial fuel cell performance by encapsulating microbial cells with a nickel-coated sponge." Biosensors and Bioelectronics **41**: 848-851.

Lu, L., et al. (2014). "Microbial Metabolism and Community Structure in Response to Bioelectrochemically Enhanced Remediation of Petroleum Hydrocarbon-Contaminated Soil." Environmental Science & Technology **48**(7): 4021-4029.

Lu, L., et al. (2014). "Enhanced bioremediation of hydrocarbon-contaminated soil using pilot-scale bioelectrochemical systems." Journal of Hazardous Materials **274**: 8-15.

Mao, D., et al. (2016). "Geophysical Monitoring of Hydrocarbon-Contaminated Soils Remediated with a Bioelectrochemical System." Environmental Science & Technology **50**(15): 8205-8213.

Morris, J. M. and S. Jin (2012). "Enhanced biodegradation of hydrocarbon-contaminated sediments using microbial fuel cells." Journal of Hazardous Materials **213**: 474-477.

Mussa, S. A. B.; Elferjani, H. S.; Haroun, F. A.; Abdelnabi, F. F. Determination of available nitrate, phosphate and sulfate in soil samples. Int. J. PharmTech Res. 2009, 1 (3), 598−604.

Wang, H.; Davidson, M.; Zuo, Y.; Ren, Z. Recycled tire crumb rubber anodes for sustainable power production in microbial fuel cells. J. Power Sources 2011, 196 (14), 5863−5866.

Wang, Xin, Zhang Cai, Qixing Zhou, Zhineng Zhang, and Cuihong Chen. Bioelectrochemical stimulation of petroleum hydrocarbon degradation in saline soil using U- tube microbial fuel cells. Biotechnology and Bioengineering 109, no. 2 (2012): 426-433.

U.S. Department of Health and Human Services. Toxicological Profile for Total Petroleum Hydrocarbons (TPH), In September 1999.

U.S. Environmental Protection Agency. Non-Halogenated Organics using GC/FID, Method 8015D. In June 2003.

Appendix A: Method Development for Total Petroleum Hydrocarbon (TPH) Measurement

Background

Petroleum hydrocarbons (PHCs) are common site contaminants. Leaking underground storage tanks (LUST) are the most frequent causes of petroleum hydrocarbon problems. Soil contamination has been a growing concern, because it can be a source of groundwater (drinking water) contamination. To monitor the pollution condition, total petroleum hydrocarbon (TPH) measurement shows that how much petroleum hydrocarbons are present in the sampled media. Measured TPH values suggest the relative potential for human health effects (ATSDR, 1999).

Petroleum products are very complex mixtures that consists hundreds of hydrocarbon compounds ranging from light, short chain organics to heavy, long-chain compounds. TPH concentration in soil samples is hard to be tested directly. It needs to be extracted out from soil samples, so can be tested through the extracts. U.S. Environmental Protection Agency (EPA) has developed a method to testing non-halogenated organics using GC/FID (Method 8015D). And Massachusetts Department of Environmental Protection has developed a method for the determination of extractable petroleum hydrocarbons (EPH). These methods give the general criterions and operation suggestions, but several different EPH extraction procedures have been approved to supplement the measurement methods. Because of the variety of samples, there is no single procedure that can be applied for all situations. The extraction process need to recover as much TPH as possible from the soil sample, meanwhile to avoid contaminations during the entire process. The purpose of developing this TPH analysis procedure is to improve the recovery during the extraction process, and calculate the TPH value appropriately from Gas Chromatograph (GC) analysis.

1.0 Supplies

- Alkane calibration standard (C8-C40, Sigma 40147-U)
- Dichloromethane (DCM)
- O-Terphenyl (OTP) (Sigma 47580-U)
- 1-Chlorooctadecane (Sigma 47584-U)
- Anhydrous sodium sulfate
- Glass centrifuge tube (35 mL)
- GasTight Syringe with polytetrafluoroethylene (PTFE) piston
- PTFE 0.45µm filter
- Glass vial (40 mL)
- Glass vial with scales (4 mL)
- GC Glass vial (2 mL)
- PTFE wash bottle

2.0 Equipment

- Vortex mixer (Fisher Scientific, Inc. USA)
- Centrifuge (Sorvall Legend X1R, Thermo Scientific, Inc. USA)
- Nitrogen evaporator (N-EVAP, Organomation Associates, Inc. USA).
- GC (Trace 1310, Thermo Scientific, Inc. USA)

3.0 Standards preparation and storage

3.1 Alkane calibration standard (C8-C40, Sigma 40147-U) is used to calibrate GC analysis. The standard with five concentrations of 200, 100, 50, 10 and 1 µg/mL is prepared using dichloromethane (DCM) as solvent. All calibration standard need to be sealed and stored at 4°C.

3.2 Surrogate is used to determine the recovery during TPH extraction process. Mixture of surrogate o-Terphenyl (OTP) (Sigma 47580-U) and 1-Chlorooctadecane (Sigma 47584-U) spiking solution with concentration of 1000µg/mL, respectively, is prepared using dichloromethane (DCM). This spiking solution needs to be sealed and stored at -20°C.

4.0 Samples collection and storage

4.1 Sampling of soils should exclude sticks, plant roots, and rocks > 2 mm.

4.2 Soil samples should be stored in a refrigerator at 4°C.

5.0 Soil dry weight Determination

The result of TPH level is normalized based on the mass of total dry soil (which is expressed as g TPH/kg dry soil. Therefore, dry weight of soil sample need to be determined.

Weigh 3g of homogenized wet soil (accurate to 0.01 g) and place in a pre-weighed aluminum pan. Dry at 105 °C in an oven for 12 hours till constant weight. Weigh the pan with soil after cool. The dry weight of pan and soil minus the weight of empty pan is the dry weight of per 3g wet soil.

6.0 Soil TPH extraction

6.1 Weigh 3g of homogenized wet soil (accurate to 0.01 g), and place into a 35 mL glass centrifuge tube. **Note**: Keep glass tube threads free of sample as this will cause the vial to leak during extraction. If needed, wipe threads with a clean Kimwipe.

6.2 Dry sample with approximately 2–3 g of anhydrous sodium sulfate till the sample is free flowing. **Note**: Be careful not to exceed half the volume of the vial. Later on, 15 mL DCM need to be added into the tube.

6.3 Using a GasTight Syringe (polytetrafluoroethylene (PTFE) piston) spike 100 µL (100 µg) of TPH surrogate mixture to the soil sample. **Note**: The final surrogate concentration in the sample extract must be within the 200 µg/mL.

6.4 Add 15 mL of DCM, securely cap the vial and invert to make sure the sample is free flowing. **Note**: All the extraction steps that involved with DCM should be operated in hood.

6.5 Vortex at maximum speed using a vortexer for 5 minutes.

6.6 Centrifuge the mixture at 3000 rpm for 5 minutes to settle all the particles in the solution.

6.7 Filter the upper-level extract using PTFE 0.45µm filter. Collect the filtered extract in a 40 mL glass vial. Securely cap the vial to prevent evaporation.

6.8 Repeat steps 4.4 through 4.6 two more times and collect all extract.

6.9 Rinse the syringe and the filter two times with 1 mL DCM each, and also collect them in the same glass vial.

6.10 Add 3-4 g of sodium sulfate into the filtered extract, cap the vial, and settle for 2 hours to remove moisture from the extract.

6.11 After 2 hours, transfer the upper clear extract to a new 40 mL glass vial. Rinse the vial and sodium sulfate 3 times with 1 mL of DCM each time

6.12 Filtered extract should be concentrated under a stream of N2 by a nitrogen evaporator (N-EVAP, Organomation Associates, Inc. USA). Samples will be placed in a 40°C water bath to enhance evaporation. Adjust the N_2 flow to form a small dimple in the solvent but not strong enough to cause splattering.

6.13 Concentrate the extract to around 2 mL. Using a glass pipette to transfer extract to a 4 mL glass vial. Rinse the 40 mL vial two times with approximately 0.5 mL of DCM each time and transfer to the 4 mL vial.

6.14 Place the 4 mL glass vial on nitrogen evaporator, and continue to concentrate and adjust extract to a 2 mL mark.

6.15 Transfer 0.8 mL extract to a 2 mL GC vial for GC/FID testing. Store the remaining extract at 4°C.

7.0 GC conditions

7.1 The GC equipped with a Restek Rxi-1 ms column (20 m x 0.18 mm ID, 0.18

μm film thickness). The GC oven temperature program will be as follows:

Injection Volume:	1 μL
Injection Port:	300 °C
GC Inlet:	Split (ratio of 1:10)
Initial Temp:	50 °C
Initial Hold:	1 min
Ramp Rate:	10 °C/min
Final Temp:	300 °C
Final Hold:	25 min
Run Time:	51 min
Carrier Gas:	Helium
Detector Air:	350 mL/min
Detector H2:	35 mL/min
Detector make up H_2:	5 mL/min
Detector Temp:	330 °C
Purge Flow:	20 mL/min
Purge Time:	1 min

7.2 Replace injection port septum and liner every 100 injections.

8.0 Calibration and Standardization

8.1 *Initial calibration* (ICAL) is the systematic determination to verify the

response of measurement system to the analyte concentration, which

should be performed before sample analysis. Alkane calibration standard

is concentrated into five concentrations: 200, 100, 50, 10 and 1 μg/mL to

calculate response factor (RF) for ICAL.

The RF of individual analyte on each standard level is calculated

using the following equation:

$$RF = (A_a/C_{std})$$

Where: A_a = Peak area of target analyte in the standard
C_{std} = Concentration of the standard

For each analyte, calculate the average RF for five levels of standards and the percent relative standard deviation (% RSD). % RSD is calculated as:

$$\%\text{RSD} = (\text{SD}/\overline{\text{RF}}) \times 100$$

Where: $\overline{\text{RF}}$ = Average response factor for each analyte
SD = Standard deviation of response factor calculated

(**Note**: For a valid initial calibration, the %RSD for each analyte should be less than 25% for 90% of the analytes and no analyte should exceed 35%.)

To calculate the average $\overline{\text{RF}}$ (RF_i) for C_{10}-C_{40} TPH from initial calibration, obtain the average of the RF for C_{10} through C_{40}, including pristane and phytane but omitting surrogates.

8.2 *Continuing calibration verification* (CCV) is subsequent checks on the instrument calibration performed throughout analysis, which is used to confirm the instrument is still within calibration.

The RF determined from the CCV is checked against those determined from the initial calibration. The percent difference between the average RF from CCV (RF_c) and the average RF from initial calibration (RF_i) is calculated using the following equation:

$$\text{Percent Difference} = [(RF_i\text{-}RF_c)/RF_i] \times 100$$

(**Note**: The percent difference should be less than 25%. Check for resolution between C_{17}/Pristane and C_{18}/Phytane which should be 75% or better. Monitor mass discrimination by comparing the ratio of the area of C40 to C20 which should not be less than 0.80.)
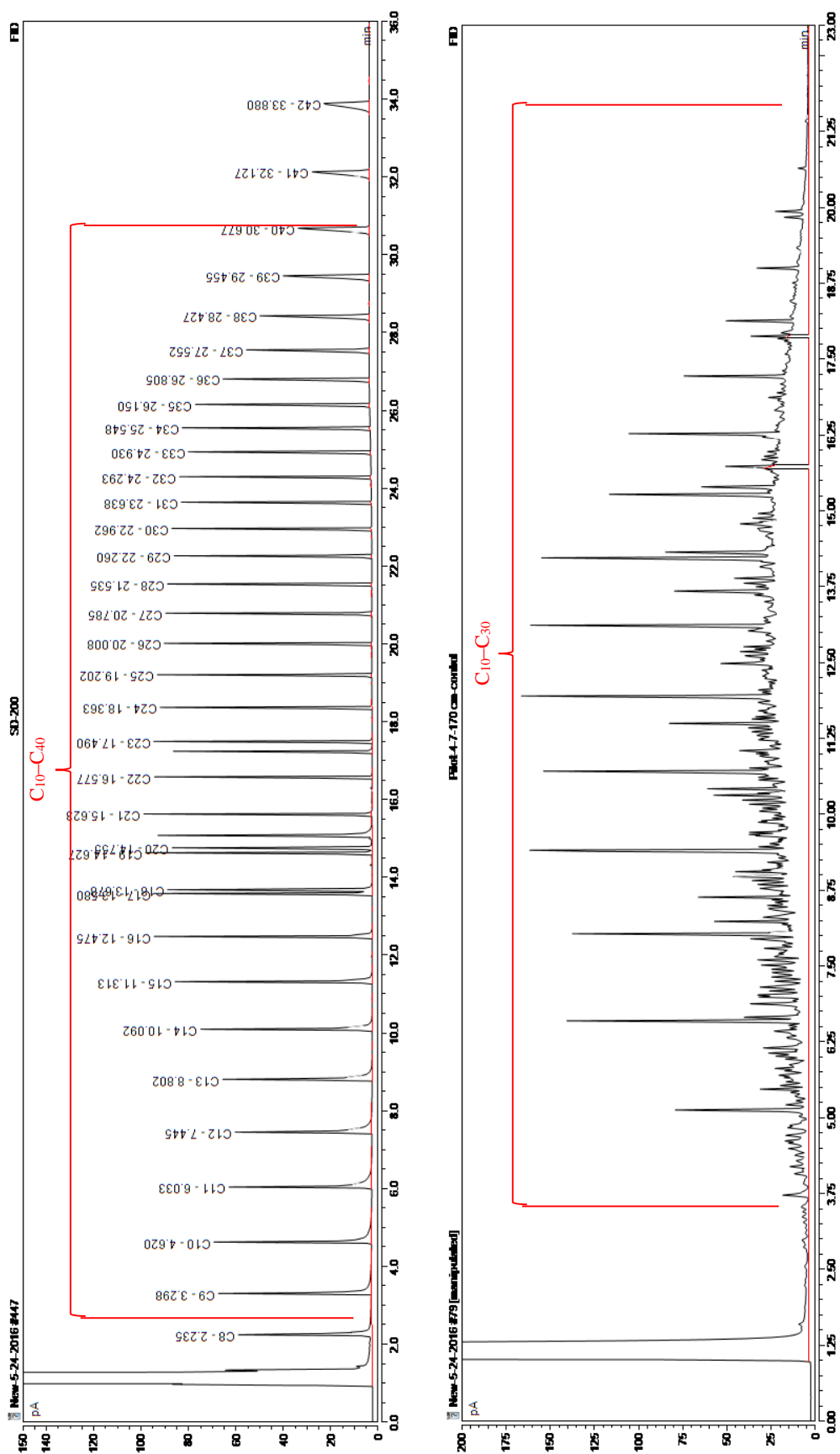
Figure 8: Left: gas Chromatogram of the Alkane calibration standard (200 µg/mL). *Right*: gas Chromatogram of a diesel contaminated soil extract.

9.0 Extraction efficiency evaluation by surrogate recovery

The extraction efficiency is evaluated by the percentage of surrogate recovery. 100 µL (100 µg) of 1000 µg/mL TPH surrogate mixture is added to the soil sample before extraction. The percentage of TPH recovery can be analyzed by the recovery of surrogate, which can be calculated using the following equation:

$$\% \text{ Recovery} = [((A_{sur}/RF_{sur}) \times FV \times D)/Q_a] \times 100$$

Where: A_{sur} = Peak area of surrogate
RF_{sur} = Average response factor for surrogate determined from initial calibration
FV = Volume of extract submitted for analysis
D = Dilution factor, if applicable
Q_a = Amount of surrogate spiked in at beginning of extraction (µg)

10.0 Quantification of Samples

Samples are analyzed under the same analytical conditions with the analytical standards. The first and the last peaks of n-alkanes ($C_{10} - C_{40}$) in the retention time window is defined as the TPH range. This retention time window should be within ±0.1 min of the standard component.

Sample dilution is needed if the height of the unresolved complex mixture (UCM) is higher than the height of the calibration.

The TPH range must be manually integrated to a horizontal integration for each sample. The area of the surrogates need to be subtracted from total TPH area.

The concentration of TPH (mg/kg-soil dry weight) in soil samples is determined using the following equation:

$$\text{TPH} = [(A/RF_i) \times FV \times D]/V$$

Where: A = Total peak area of TPH range of $C_{10} - C_{40}$

RF_i = Average RF for $C_{10} - C_{40}$ determined from initial calibration

FV = Volume of extract submitted for analysis
D = Dilution factor, if applicable
V = Dry weight of soil sample

11.0 Quality control

11.1 For each sampling, two soil samples are collected from each sampling location as laboratory duplicates. Criteria: < 30% RSD.

11.2 Before TPH extraction, surrogate is added to the sample to evaluate the extraction efficiency. Criteria: 70–130% recovery.

11.3 To monitor laboratory contamination, a procedural blank is added for each batch sample analysis. Sodium sulfate and surrogate are spiked with DCM in the procedural blank, and are carried through the extraction procedure. Any UCM appeared in procedural blank other than DCM or surrogate is considered as laboratory contamination. UCM from laboratory contamination need to be omitted from TPH quantification, TPH extraction process need to be repeat if necessary.

11.4 During ICAL, mid-level calibration standard 50 µg/mL is made from a second source using either a different lot number or different vender. This is considered as subsequent second source calibration. Criteria: %RSD < 25% for 90% of analytes and < 35% for all analytes; 80–120% recovery.

11.5 An instrument blank should be run before each analytical sequence. The area of the instrument blank is subtracting from the area of the sample during sample integration, so that the area of baseline drift can be removed. This can leave only TPH contributions in the sample integration.

11.6 CCV is conducted at the beginning and the end of each sequence. Criteria: %RSD < 25%.

11.7 Check for resolution between C_{17}/Pristane and C_{18}/Phytane which should be 75% or better for each CCV.

11.8 Monitor mass discrimination by comparing the ratio of the area of C40 to C20 which should not be less than 0.80.

11.9 The system must be recalibrated if significant maintenance is performed, i.e. FID jet cleaning, column changing.

12.0   Troubleshooting

12.1 The soil sample get caking or stuck to the bottom after adding anhydrous sodium sulfate.

12.2 This happens when soil has high moisture level. To solve this problem, add 10 mL of DCM and shake to break up. Use a clean spatula to break up the sample if necessary, then rinse the spatula with 5 mL DCM.

12.3 The Alkane calibration standards with high concentrations have flocks under 4°C stored.

12.4 This happens especially to the 200 and 100 µg/mL standers. So before place standers on GC for testing, set them in 35°C incubator for 10 mins. After taking them out from the incubator, use hands to keep them warm and shake them well before injection.

Appendix B: Graphite granule to hydrogel ratio test

Graphite granule and hydrogel mixture is using as water retention layer for GHB reactor. Five different ratios are tested to determine the most appropriate one to apply in the reactor. Three properties were tested for each mixture: moisture level change, resistance change, and volume change through time. For moisture level test, the longer the mixture can hold the moisture, the better. For resistance test, the smaller the mixture resistance is, the better. For volume test, the less the volume of mixture shirks, the better. 45 mL of each mixture was added into open cap centrifuge tube for evaporation. By the end of the 12-days evaporation test, the moisture levels of mixture decrease as the hydrogel percentage in the mixture decrease. The resistances of the mixture increase as the hydrogel percentage in the mixture increase. The volume of the mixtures decreased more in the one with more hydrogel content. Among the three properties that were tested, G:H 2:1 showed the best result on two of them (smallest resistance and smallest volume change) compare to G:H 1:2 and GH 1:1. Therefore, G:H 2:1 was selected the ratio been applied to the reactor.
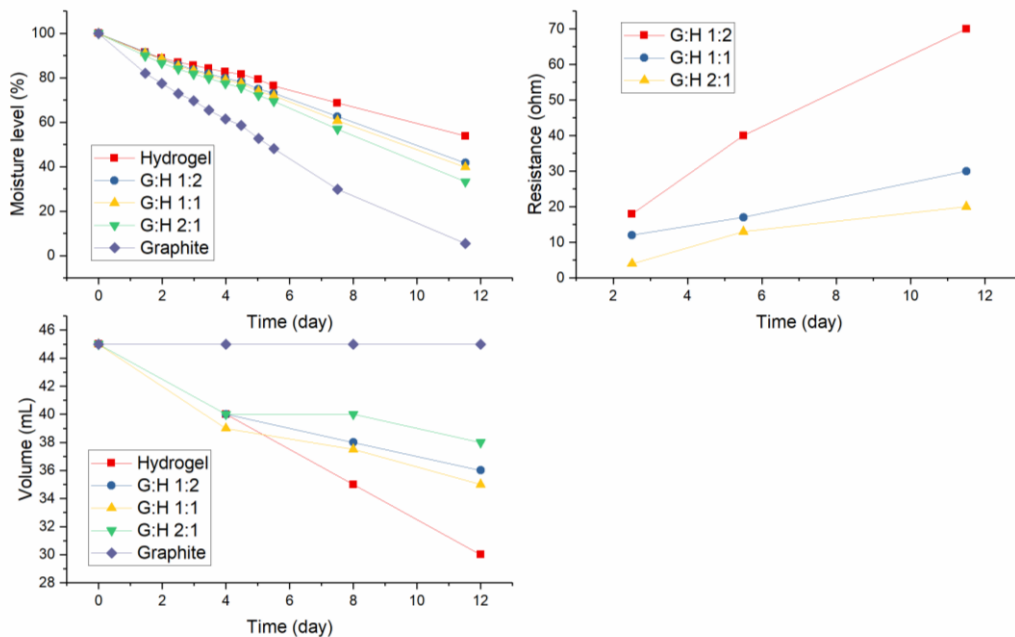

 Figure 9. moisture level change, resistance change, and volume change of different graphite granule to hydrogel ratios through the time.