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Bio-electrokinetic remediation of crude oil contaminated soil enhanced by bacterial biosurfactant

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ABSTRACT

The present study evaluating the coupling between bioremediation (BIO) and electrokinetic (EK) remediation of crude oil hydrocarbon by using bio-electrokinetic (BIO-EK) technique. The application of bacterial biosurfactant (BS) may increase the remediation efficiency by increasing the solubility of organic materials. In this work, the potential biosurfactant producing marine bacteria were isolated and identified by 16S rDNA analysis namely *Bacillus subtilis* AS2, *Bacillus licheniformis* AS3 and *Bacillus velezensis* AS4. Biodegradation efficiency of crude oil was found as 88%, 92% and 97% for strain AS2, AS3 and AS4 respectively, with the optimum temperature of 37 °C and pH 7. FTIR confirm the BS belongs to lipopeptide in nature. GCMS reveals that three isolates degraded the lower to higher molecular weight of the crude oil (C_8 to C_{28}) effectively. Results showed that use of BS in electokinetic remediation enhance the biodegradation rate of crude oil contaminated soil about 92% than EK (60%) in 2 days operation. BS enhances the solubilization of hydrocarbon and it leads to the faster electromigration of hydrocarbon to the anodic compartment, which was confirmed by the presence of higher total organic content than the EK. This study proven that the BIO-EK combined with BS can be used to enhance in situ bioremediation of petroleum contaminated soils.

1. Introduction

Large scale production of crude oil and its transportation leads to huge chances for oil spills in soil, and sea water environments (Nriagu, 2011). The major parts of crude oil are hydrocarbon with low and high molecular weights and they are accessible by many microorganisms including bacteria, fungi, yeast, and archaea (Varjani, 2017). The microorganisms involved in various complicated pathways in the biodegradation process by producing key molecules that are acting as synergistic intermediates to enhance the biodegradation process in electrokinetics (Rajasekar and Ting, 2010; Li et al., 2012; Gill et al., 2014; Elumalai et al., 2017). Synthetic surfactants such as cetyltrimethyl ammonium bromide (cationic), sodium lauryl sulphate (anionic), phospholipids phosphatidyl serine have been widely used in the oil industry for oil spills clean-up process, enhanced oil recovery, but problem associated with these synthetic surfactants is their toxicity and non-biodegradability (Lima et al., 2011). Alternative options to overcome these problems are biosurfactant (Perfumo et al., 2010). Biosurfactants are surface-active molecules produced by fungi, bacteria and yeast and produced mostly as extracellular products during the microbe's growth in starvations and hyper environments including pH, temperatures and nutrient depletions (Naeem and Qazi, 2019). The biosurfactant can be classified into several types including lipopeptides, phospholipids, glycolipids, natural lipids, lipopolysaccharide, and fatty acids.

Biosurfactant is having numerous advantages like environmentally friendly, biodegradable, stable at higher temperature and pH, can be synthesized using inexpensive raw material and non-hazardous

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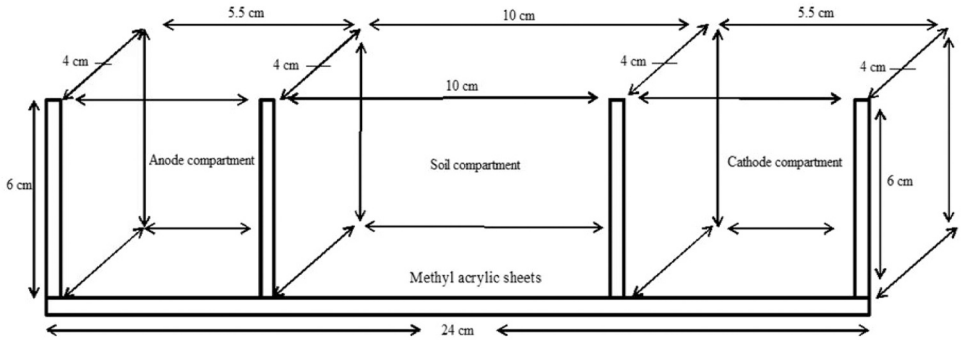

Fig. 1. Designing of electrokinetic cell tank.

(Virkutyte and Varma, 2011). This feature makes cheap and simple production of biosurfactant with industrial and agricultural waste substrates and in turn which reduces waste and contaminants indirectly (Campos et al., 2013; Parthipan et al., 2017a; Parthipan et al., 2018a, 2018b; Tan and Li, 2018). Biosurfactant having an aptitude to accrued at the interface of two immiscible liquids or between a liquid or solid (Sadouk et al., 2008). By reducing the surface and interfacial tension, they dropping the repulsive forces among two different stages and permit them to blend and intermingle more simply (Patowary et al., 2017). Thermophilic structure of biosurfactant increases the surface area of hydrophobic water-insoluble substances and increases bioavailability, which will assist in biodegradation by microorganisms (Parthipan et al., 2018a). These surface activities make surfactant as excellent emulsifiers, foaming, and dispersing agents (Lim et al., 2009).

Electrokinetics (EK) is a method to separate the heavy metals in solid waste or solid matter (Rajasekar et al., 2010; Huang et al., 2018). Limited direct current (DC) is applied to electrodes and extraction of metals based on the charges. EK removal of a pollutant has established as a potential approach for cleaning up heavy metal polluted soils (Khalid et al., 2017). The outcome of the electrokinetic remediation is to effective separation of molecules based on electroosmosis, electromigration, and electrophoresis (Park et al., 2010). In addition to physical and biological factors, electrochemical factors are having a key impact on the bioremediation rates (Agu and Okoli, 2014). The efficacy of Eh measurements, Eh-pH illustrations, and galvanic relations in bioremediation reactions have been well recognized (Verma and Kuila, 2019). The sluggishness of the bio-oxidation process and the real-time application problems related to the collecting of adequate levels of the biomass of the microorganisms have habitually restricted the scope and broader application of bioremediation (Chandra and Chowdhary, 2015). It has been revealed that the cell biomass of specific stains and their activity might be improved the electrochemical reactions. The electro-bioremediation technique has the advantage of being able to simultaneously increasing the degradation rates and cell yield in the hydrocarbon polluted environment (Koshlaf and Ball, 2017; Verma and Kuila, 2019).

In this work, we have collected the seawater sample from mangroves for the isolation of biosurfactant synthesizing bacterial species. Isolated marine bacterial strains were identified by 16S RNA gene sequencing. All the isolated strains are screened for biosurfactant production and subjected to optimization of biosurfactant production by alteration of pH and temperature. Extracted biosurfactant were analysed using gas chromatography and mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy (FT-IR) analysis. Further, the purified biosurfactant was applied for biodegradation of crude oil by using bio-electrokinetic approach.

2. Materials and methods

2.1. Sample collection

The seawater sample was collected from mangroves forest at Pichavaram, Tamil Nadu, India (latitude-11.4319° N and longitude-79.7810° E (Fig. S1). Sample was collected using sterile sample containers and kept in an icebox and immediately transported to lab.

2.2. Isolation and identification of bacterial strains

For isolation of marine bacteria from seawater samples, the standard serial dilution method and pour plate technique were employed by using zobell marine medium (Hi-media, India) for this isolation of bacteria and the inoculated plates were incubated at 37 °C for 24 h. Distinct colonies by appearance, shape, colours were selected for further identification. The repeated streaking plate method was carried out for the isolation of a single colony without contaminants (Sosa et al., 2017). Further, isolated strains were subjected to biochemical characterization as described in Bergey's Manual of Systematic Bacteriology (Christensen and Martin, 2017). Further Molecular identification of selected strains is done using the universal primer as described in (Rajasekar et al., 2010). After the successful sequencing, all the obtained sequence is aligned/edited before submission into the National Centre for Biotechnology Information (NCBI) GenBank (www.ncbi.nlm.nih.gov). ClustalW and MEGA (version 6) software was used to build a phylogenetic tree by the neighbour-joining method (Tamura et al., 2013).

2.3. Screening and optimization, production and characterization of biosurfactant

All the isolated bacterial strains were subjected to the screening for biosurfactant production by different screening methods. A sterile Zobell marine medium was prepared and supplemented with 2% of crude oil (0.2 μm filter sterilized) as a single carbon basis. Isolated bacterial strains were pre-cultured and inoculated into the growth medium and kept in a shaking incubator for 120 h at 37 ºC in a shaking incubator at 150 rpm. At the end of the incubation, the supernatant was collected by separating the biomass using centrifugation at 5000 rpm for 30 min at 4 ºC. Further, this supernatant was utilized for screening tests such as drop collapse assay and emulsification assay as described in (Patowary et al., 2017; Parthipan et al., 2017a) oil spreading assay as mentioned in (Hassanshahian et al., 2014).

Further, three strains such as AS2, AS3, and AS4 were selected for the optimization of their growth to enhance their biosurfactant producing capability. For this purpose, pH (4–10) and temperature (10–60 °C) were optimized based on their biomass level by growing them in specific conditions as specified in biosurfactant screening. The optimized conditions are applied for the effective production of biosurfactant. Finally, these extracted and partially purified biosurfactant was subjected to the

FT-IR (Perkin–Elmer, Nicolet Nexus – 470) analysis to confirm their functional groups using Kbr in the mid-IR region 400– 4000 cm^{-1}. Similarly, GC-MS (Shimadzu QP2010 Ultra, Rtx-5Sil MS) analysis was used for the structural confirmation of specific biosurfactant molecules obtained from each bacterial strain as specified by Parthipan et al. (2017a).

2.4. Design and fabrication of electrokinetic cell for hydrocarbon degradation

The EK cell was fabricated with methyl acrylic sheets, which was separated into three compartments (24 × 4 × 6 cm, L x W x H). The soil sample was collected from Thiruvalluvar University campus, Serkadu, Vellore district. The soil was sieved (2 mm) prior to use. The hydrocarbon contaminated soil was prepared by mixing 2% of sterile crude oil into the soil samples and cautiously placed in the central chamber (Steager et al., 2011). Anolyte and catholyte reservoirs were filled with sterile distilled water as a working solution. IrO_2–RuO_2–TiO_2/Ti and Ti plate were served as anode and cathode, respectively. Direct current (DC) supply was used for impressing constant voltage/constant current. The maximum voltage gradient was 2 V/cm^2 for effective elimination of organic and inorganic components in the EK system (Rodrigo et al., 2014). The entire EK study was carried out for 2 days. After that, soil was divided into five sections from the anode to cathode. Biosurfactant (0.5 W/V) solution of each bacterial strain was added in to catholyte compartment (Fig. 1). Besides, the uninoculated BS served as control EK. The residual crude oil absorption was analysed during Bio-EK was characterized by GCMS and the total organic content was also estimated.

2.5. Analytical method

2.5.1. Gas chromatography mass spectroscopy (GCMS)

The residual crude oil obtained after an electrokinetic study was subjected to GCMS analysis to confirm the quantitative degradation level of various hydrocarbons present in the crude oil samples. GCMS analysis was carried out as described above. The degradation of organic carbon present in the crude oil was assessed during the BIO-EK by GCMS. Besides, the uninoculated biosurfactant served as control EK.

2.5.2. Analysis of total organic carbon

For the characterization of total organic carbon (TOC) of samples, 0.5 g of soil sample was weighed and (passed through 0.2 mm sieve) transferred to 500 mL conical flask (Cang et al., 2009). Further 10 mL of 1 N $K_2Cr_2O_7$ was included and mixed well by swirling the flask. Further 20 mL of sulphuric acid was included and mixed by moderate rotation for 1 min to confirm complete mixing contents of the reagent. This setup was allowed to stand for 20–30 min and keep an asbestos sheet to avoid overheating. After 30 min, 200 mL of deionized water was included and 10 mL of 85% of phosphoric acid was included followed by 1 mL of diphenylamine indicator was added (Cang et al., 2009). Further, the solution was subjected to titration with 0.5 N of ferrous ammonium sulphate. The colour is dull green at the beginning and then shifted to turbid blue as the titration proceeds, the end point is bright green colour. The same protocol was followed for blank without soil. A volume of 0.5 N of ferrous ammonium sulphate consumed was noted for the calculation of TOC (Wang et al., 2004).

3. Results and discussion

3.1. Isolation and identification of marine bacteria

The seawater sample was used to isolate marine bacterial strains with the crude oil-degrading ability. About seven dissimilar colonies were isolated and pure strains are used for the biosurfactant screening. Based on the preliminary screening three bacterial strains are selected as efficient biosurfactant producers among the bacterial strains.

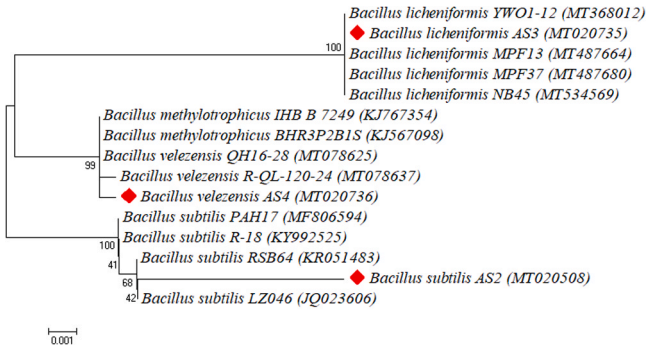

Fig. 2. Phylogenetic tree of bacterial strains of *B. subtilis* AS2, *B. licheniformis* AS3 and *B. velezensis* AS4.

Table 1
Screening for biosurfactant production of the marine bacterial isolates.

Strains	Oil spreading	Drop collapse	Emulsification index %	Biomass of biosurfactant (mg/l)
AS1	++	+	51 ± 1	0.312
AS2	+++	+++	77 ± 2	0.563
AS3	+++	+++	74 ± 2	0.355
AS4	+++	+++	68 ± 2	0.517
AS5	++	+	62 ± 1	0.268
AS6	+	++	44 ± 1	0.480
AS7	++	++	58 ± 1	0.372

Biochemical characterization revealed that all the isolated bacterial strains are Gram-positive and also belongs to *Bacillus* species (Table S1). For the confirmation of bacterial species 16S rDNA, gene sequencing was carried out. Molecular identification revealed that different species of *Bacillus* only found in the seawater sample. Those biosurfactant producing bacterial strains AS2, AS3, and AS4 were identified as *Bacillus subtilis* AS2, *Bacillus licheniformis* AS3, and *Bacillus velezensis* AS4 respectively. The aligned 16S rDNA gene sequence was submitted in National Centre for Biotechnology Information and obtained accession numbers as MT020508 (*B. subtilis* AS2), MT020735 (*B. licheniformis* AS3) and MT020736 (*B. velezensis* AS4) respectively. The phylogenetic relationship of these strains with relevant bacterial strains was illustrated in Fig. 2. Phylogeny analyses reveal the evolutionary development of group of bacteria and are determined by biochemical and genetic characteristics.

3.2. Screening of biosurfactant

Primary and secondary screening methods are used to choose efficient bacterial strains as specified in Table 1. In an oil spreading study, a clear zone with a range of 1.5–2 cm was considered as the most efficient biosurfactant producers. As shown in Supplementary Fig. S2, strain AS2, AS3, and AS4 are met at this level of oil spreading with great potential (Korayem et al., 2015). Other bacterial strains such as AS1, AS5, AS6, and AS7 are showed less oil spreading activity only. Similar activity was found with the drop collapse study with quick collapse with strain AS2, AS3, and AS4 as shown in Supplementary Fig. S3. Further, emulsification activity (E24) of the bacterial strains is checked for all strains and found that 77%, 74%, and 68% for strains AS2, AS3, and AS4 respectively. E24 was a very important parameter to choose a bacterial strain with higher efficiency for the immiscible pollutant's degradations (Plaza et al., 2006). These screening methods revealed that three strains including AS2, AS3, and AS4 had higher biosurfactant producing aptitude. Recently, Parthipan et al. (2017a) reported that *B. subtilis* A1 as an efficient biosurfactant producing bacterial strains, which also showed significant crude oil biodegradation activity.

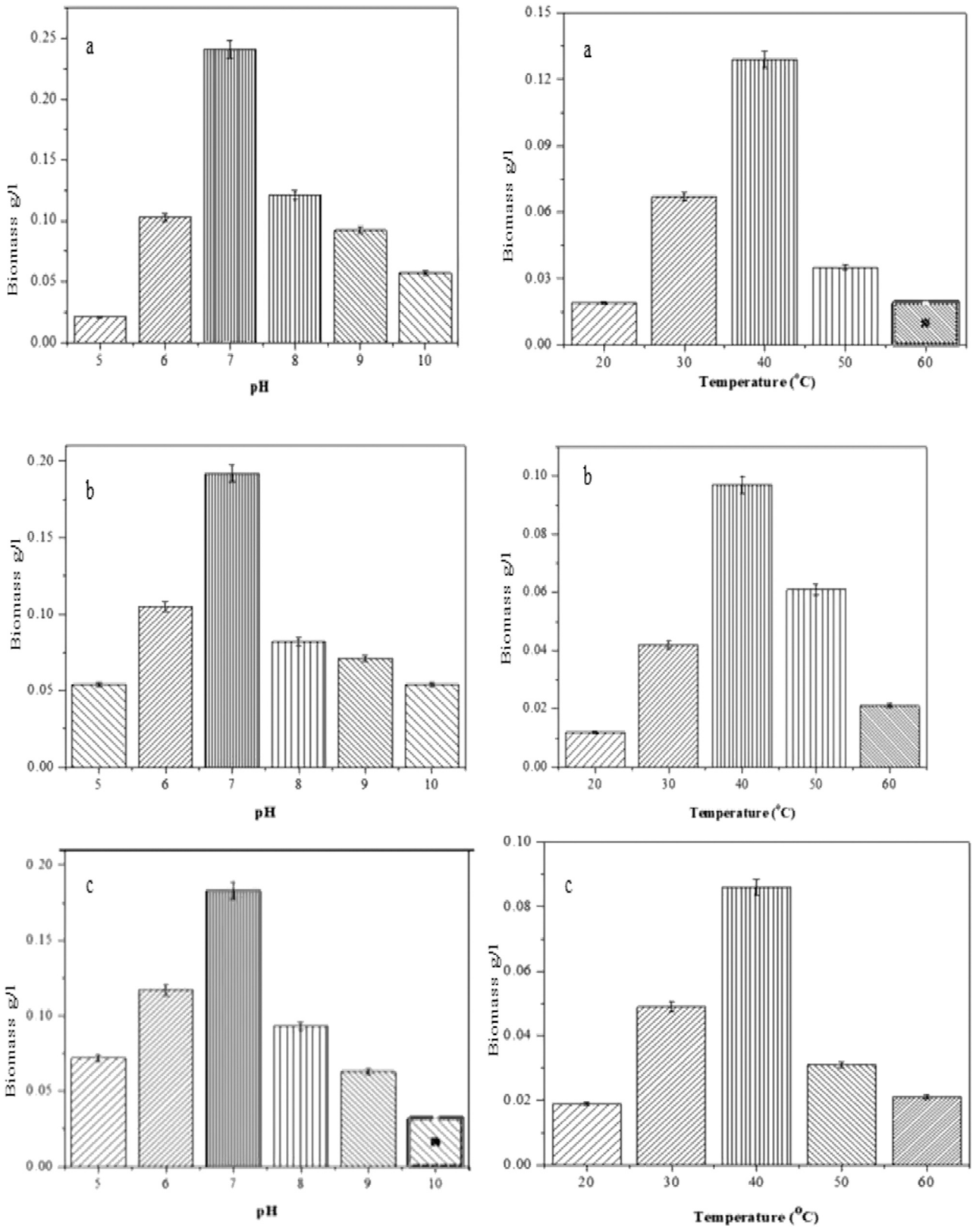

Fig. 3. Effect of pH and temperature on growth of biosurfactant producing bacterial strains: *B. subtilis* AS2 (a, b), *B. licheniformis* AS3 (c, d) and *B. velezensis* AS4 (e, f) respectively.

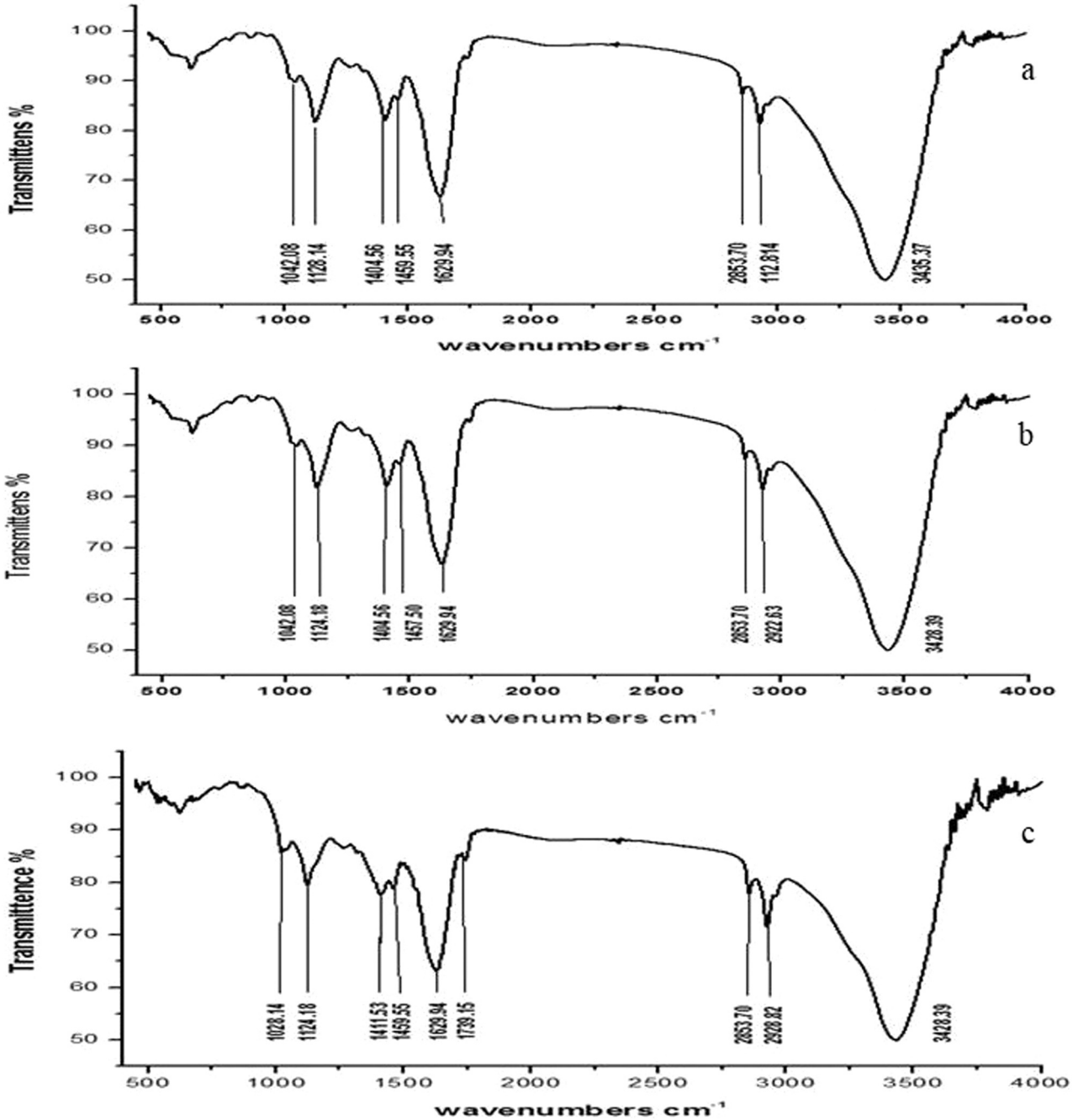

Fig. 4. Fourier transform infrared (FT-IR) spectrum of purified biosurfactant produced by the selected isolates, (a) *B. subtilis* AS2, (b) *B. licheniformis* AS3, (c) *B. velezensis* AS4.

3.3. Optimization of growth parameters

Biosurfactant screening results confirmed that three strains such as *B. Subtilis* AS2, *B. licheniformis* AS3, and *B. velezensis* AS4 are efficient biosurfactant producers. Further, these selected three strains are subjected to the optimization of pH and temperature to confirm the impact of pH and temperature changes in their growth and development as illustrated in Supplementary Table S2. Different pH and temperature were optimized in this study. The biosurfactant production with different pH and temperature was expressed as increases in bacterial biomass. The biosurfactant containing culture broth was centrifuged and the pellet was used for the measurement of biomass. As mentioned in Fig. 3, bacterial development and growth were severely reduced at pH 5.0 and 6.0. Usually, low pH leads to an inauspicious situation for all the bacterial strains (Khopade et al., 2012). Expectedly, pH 7.0 was confirmed as optimum pH for all three strains, there is no surprise with this outcome since most of the marine microorganisms grow very well in neutral pH (pH: 7–8). When the pH was set as more than 8.0, a sharp decline in the growth was observed (Fig. 3).

Another physiochemical factor that massively alters the growth and development of any microbial strains was growth temperature and results obtained for optimization of temperature were presented in Fig. 3. All three bacterial strains showed 40 °C as an optimal temperature for their growth and development. Low temperature 10–20 °C was not suitable for the growth and development, many of the metabolism could be in trouble at low temperature and some of the biochemical reactions might be inactive or improper (Parthipan et al., 2017a). Higher temperature i.e 50–60 °C was not ideal temperature for many bacterial

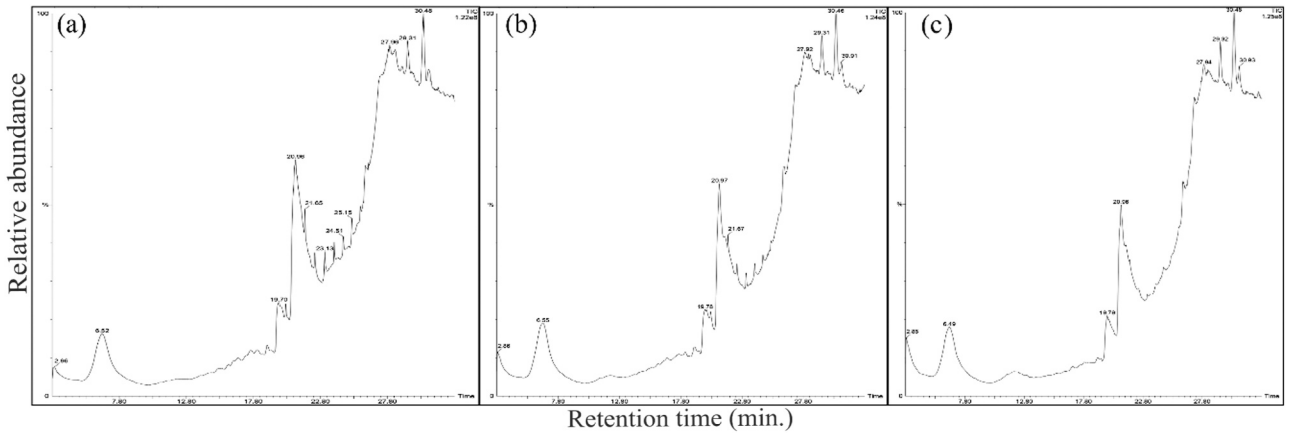

Fig. 5. Gas chromatography analysis of purified biosurfactant produced by the selected isolates, (a) *B. subtilis* AS2, (b) *B. licheniformis* AS3 and (c) *B. velezensis* AS4.

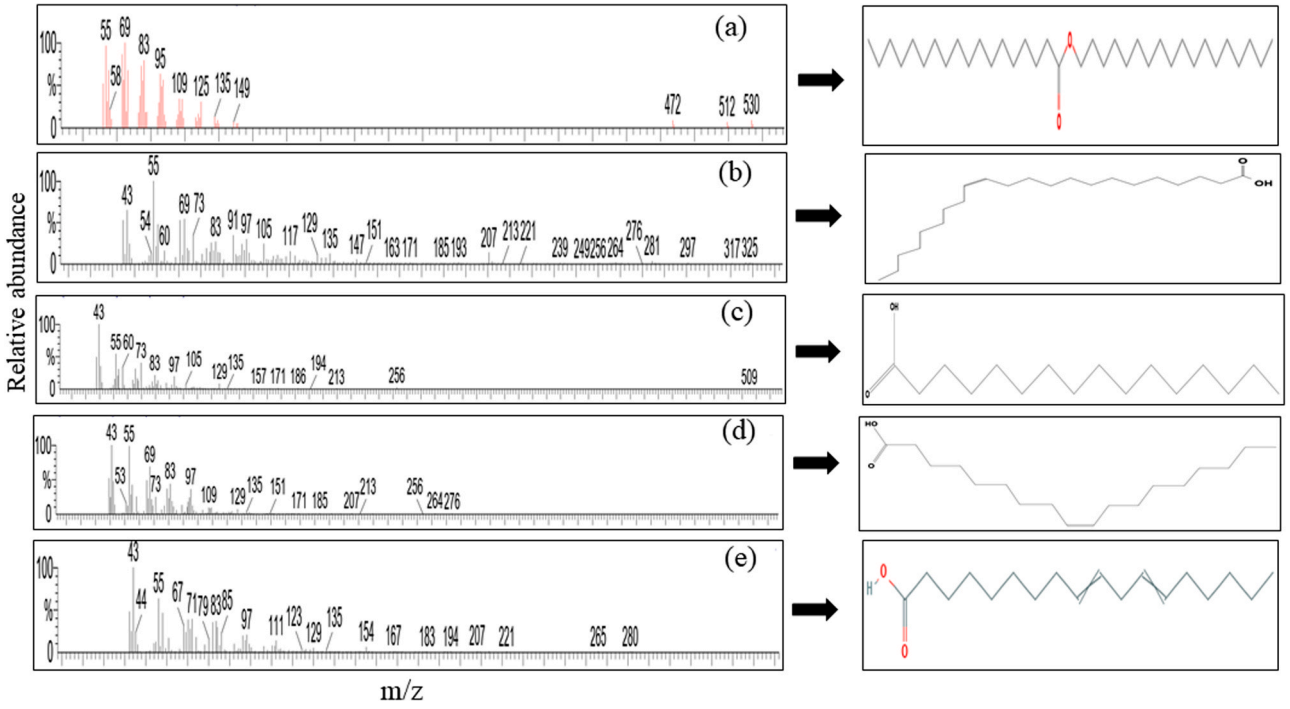

Fig. 6. Mass spectra of purified biosurfactant molecules (a) octadecanoic acid, octadecyl ester, (b) erucic acid, (c) n-hexadecanoic acid, (d) oleic acid, and (e) 12-octadecadienoic acid (z,z) .

strains except which have the thermophilic characteristics. For mesophilic bacteria, higher temperature makes them very difficult for survival (Parthipan et al., 2017b). If the temperature raised, which will lead to a low to moderate reduction in their biomass as shown in Fig. 3 (Parthipan et al., 2017a). Biosurfactant has proven surface active molecules to clean the hydrocarbon polluted environment and are cheaper and eco-friendly techniques (Koshlaf and Ball, 2017; Parthipan et al., 2017b).

3.4. Characterization of biosurfactant

The FTIR spectrum of a biosurfactant obtained from strain AS2 found important bands at 3435 cm^{-1} which represent that -OH. A distinct peak at 2853 cm^{-1} indicated that the presence of –C–H cm^{-1} stretching mode of CH_3 and CH_2 groups in alkyl chains. A peak at 1629 cm^{-1} confirms the existence of CO–N stretching vibration. The weak band in the range of 1370–1470 cm^{-1} indicates that deformation and bending vibrations of –C–CH_2 and –C–CH_3 groups in aliphatic chains (Fig. 4a). Similar functional groups only found in the biosurfactant obtained from AS3 and AS4 as well (Fig. 4b and c respectively). Biosurfactant obtained from other *Bacillus* species also shows similar functional groups. (Pornsunthorntawee et al., 2008; Ismail et al., 2013; Parthipan et al., 2017a).

Further, extracted biosurfactant was subjected to the Gas chromatography analysis and presented in Fig. 5. The mass spectrum and their respective chemical structure obtained for the major retention peak of all three bacterial strains are presented in Fig. 6. From this figure, it was very clear that all three strains are produced fatty acids type of biosurfactant. In specific, strain AS2 produced octadecanoic acid, octadecyl ester (Fig. 6a and b) ($C_{36}H_{72}O_2$, Molecular weight (MW): 536) (Sharma et al., 2014) and erucic acid ($C_{22}H_{42}O_2$; MW: 338). Similarly, strain AS3 produced, n-hexadecanoic acid ($C_{16}H_{32}O_2$; MW: 256) (Fig. 6c) (Sharma et al., 2015) and oleic acid (Fig. 6d) ($C_{18}H_{34}O_2$; MW:282) (Parthipan et al., 2017a). Similar to the strain AS2 and AS3, strain AS4 produced oleic acid and erucic acid. In addition to these two fatty acids, 9,12-octadecadienoic acid (z,z)- (Fig. 6e) also synthesized ($C_{18}H_{32}O_2$; MW: 280). Mass spectra analysis revealed that all three bacterial strains were

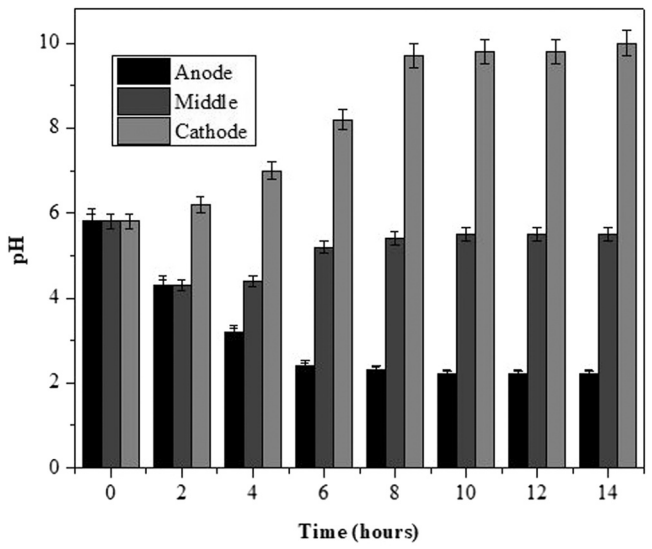

Fig. 7. pH changes of soil during the electrokinetic study.

producing fatty acids types of biosurfactant. Gas chromatography analysis strongly supports the data obtained using the FT-IR spectrum that, all the *Bacillus* strains are producing lipopeptide types of biosurfactant (Parthipan et al., 2017a).

3.5. Bio-electrokinetic remediation of crude oil contaminated soil

3.5.1. pH changes during the electrokinetic reactions

During the electrokinetic study, the pH of the soil was playing a key role in the electrokinetic process as illustrated in Fig. 7. From this figure, anodic sites are clearly shown that more acidic conditions for anode soil in the range between pH 2.0–4.0. Due to the generation of H^+ ions from that anodic reaction, whereas in the middle section pH ranges between pH 4.0–6.0 cathodic sites were observed that neutral to alkaline condition with a pH range of 6.0–10.0. From this observation, it believed that the acidic condition of the soil was favourable for the electrokinetic technique to achieve the effective crude oil degradation.

3.5.2. Total organic carbon (TOC) analysis

After the EK experiment, the total organic content (TOC) of the soil samples, anode, and cathode section calculated were 87%, 94%, and 62% respectively (Table 2). In the appearance of biosurfactant mediated electrokinetic remediation 37%, 82%, and 9% respectively. The removal of TOC was higher in presence of BS due to the higher solubilization of hydrocarbon and its movement to the anode compartment, which was due to the negatively charged molecules/hydrocarbon, which resulted in faster degradation of hydrocarbon evidenced from the increasing values of the TOC in the anode and soil section when compared to EK. The volume of organic carbon attained showed variances among the studied methods and, as anticipated, highly associated with each order, which was in supported with the literature (Bianchi et al., 2009). The highest correlation coefficient with the reference met.

3.5.3. Gas-chromatography analysis of degraded crude oil

Biodegradation of crude oil was done by an integrated approach using electrokinetic technology with the aid of biosurfactant to solubilize the complex crude oil into simple hydrocarbons (Gomes et al., 2012). The bioremediation process was continuously monitored, initially; soil samples (light colour) were contaminated with 2% of crude oil (dark brown colour) and subjected to the electrokinetic remediation with the addition of the biosurfactant. This remediation process was continued to 2 days. At the end of the remediation process, surprisingly soil turns back to its natural colour (light colour) (Fig. S4). At the end of the biodegradation period, residual crude oil in the soil samples was collected and subjected for the GCMS analysis as presented in Fig. 8. The biodegradation efficiency of crude oil by *B. subtilis* AS2, *B. icheniformis* AS3, and *B. velezensis* AS4 was about 88%, 92% and 97% respectively.

More specifically, as illustrated in Table 2, most of the low molecular weight hydrocarbons present in the crude oil samples are completely utilized by all three bacterial strains. Hydrocarbons vary from C_8 to C_{28} in typical crude oil sample among these C_8–C_{15} such as 2-piperidinone, oxalic acid, nonadecane, sulphurous acid, tricosane, heptacosane, hexatriacontane, hexatriacontane, and triacontane were entirely utilized by all the three strains. Residual hydrocarbons such as octacosane, tritetracontane, tetratetracontane, and dotriacontane were degraded by 60–90%. From gas chromatography analysis it was very clear that these bacterial strains having the aptitude to degrade both low and high molecular weight of hydrocarbons existing in the crude oil samples in a short time (Parthipan et al., 2017a).

The combination of bioleaching and EK may demonstrate to have synergistic characteristics (Maini and Taylor, 2000; Pazos et al., 2010). In particular, EK transports metal ions on the condition that their speciation is suitable. Metals as oxides or hydroxides may be solubilized by the EK acidification with the production of biosurfactant, thus

Table 2
Biodegradation efficiency of crude oil the in presence of biosurfactant produced by marine bacterial strains.

Compounds	MW	Formula	RA Control	BS-AS2	BE %	BS-AS3	BE %	BS-AS4	BE %
2-piperidinone	233	C_5H_9NO	40.4	0	100	0	100	0	100
Oxalic acid	314	$C_{18}H_{34}O_4$	48.7	0	100	0	100	0	100
Nonadecane	302	$C_{19}H_{39}C_l$	54.5	0	100	0	100	0	100
Sulphurous acid	404	$C_{23}H_{48}O_3S$	80.5	0	100	0	100	0	100
Tricosane	338	$C_{24}H_{50}$	60.7	0	100	0	100	0	100
Heptacosane	414	$C_{27}H_{55}C_l$	64.4	0	100	0	100	0	100
Octacosane	394	$C_{28}H_{58}$	65.6	9.4	90.6	0	100	0	100
Dotriacontane	450	$C_{32}H_{66}$	80.5	0	100	22.8	77.2	9.4	90.6
Hexatriacontane	506	$C_{36}H_{74}$	94	0	100	55.7	42.3	49.1	50.9
Tritetracontane	604	$C_{43}H_{88}$	99.2	38.7	61.3	41.4	58.6	0	100
Tetratetracontane	618	$C_{44}H_{90}$	94.3	48.6	51.4	0	100	0	100
Triacontane	702	$C_{50}H_{102}$	74.3	0	100	0	100	0	100
Triacontane	702	$C_{50}H_{102}$	70.6	37.9	62.1	0	100	0	100
Triacontane	702	$C_{50}H_{102}$	65.2	0	100	0	100	0	100
Triacontane	702	$C_{50}H_{102}$	63	41.7	58.3	0	100	0	100
Triacontane	702	$C_{50}H_{103}$	51.6	46.6	53.4	0	100	0	100
Triacontane	702	$C_{50}H_{104}$	44.1	0	100	0	100	0	100
Triacontane	702	$C_{50}H_{104}$	31.8	0	100	26	74	0	100
Total percentage					88		92		97

Note: MW-Molecular weight, RT = Retention time, RA = Relative abundance (%), BS-Biosurfactant, AS2-Biosurfactant from *B. subtilis*, AS3 = Biosurfactant from *B. licheniformis,* AS4-Biosurfactant from *B. velezensis*

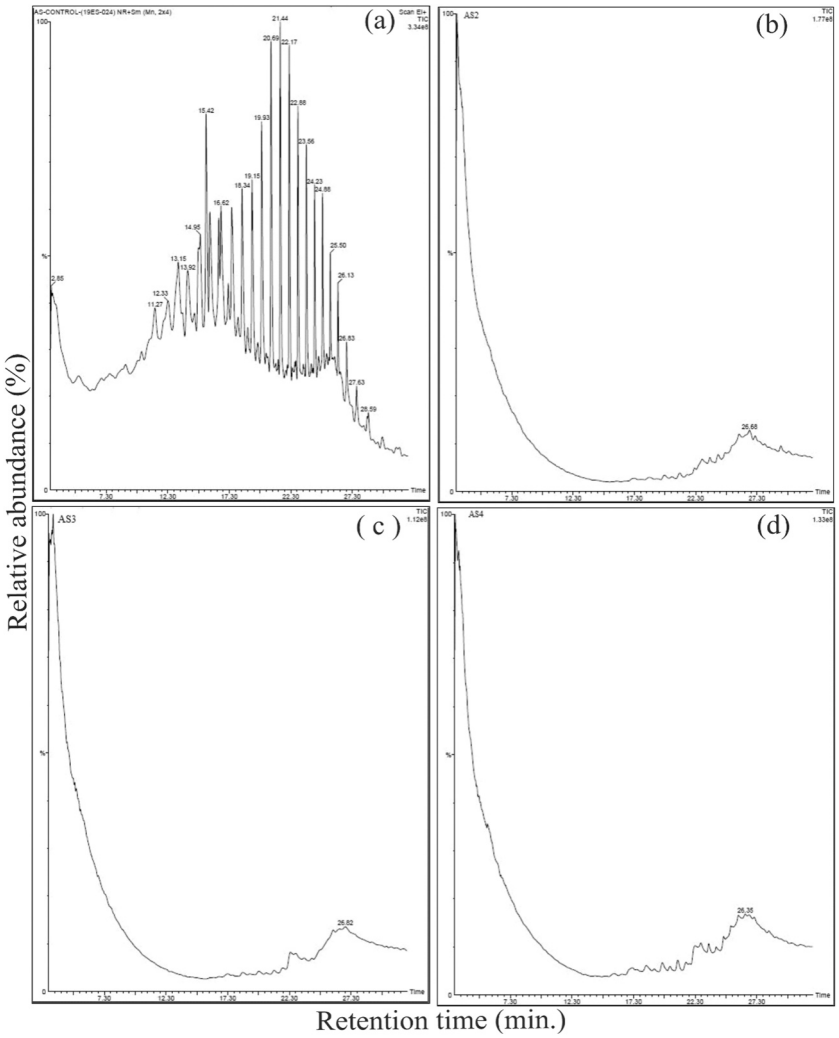

Fig. 8. Gas chromatography analysis of crude oil degradation (a) Control crude oil (b) *B. subtilis* AS2, (c) *B. licheniformis* AS3 and (d) *B. velezensis* AS4.

permitting their solubilization and succeeding transportation by electromigration to anode as illustrated in (Fig. S5). In count, the directional transport of charged hydrocarbon ions by EK is a suitable accompaniment to bioremediation as solubilized hydrocarbon can be removed at the anode for straightforward removal of pollutants (De-Bashan and Bashan, 2010). A further benefit of combining bioleaching with EK is the potential to decrease the total time scale and cost of EK remediation (Rodrigo et al., 2014). As reported earlier, the biosurfactant is negatively charged and readily adsorbed onto the serpentinite surface, resulting in zeta potential changes and resulted in the solubilization of hydrocarbon. This combination reaction of biosurfactant with hydrocarbon causes the negatively charged molecules and thus moves to the anode. The adsorption of biosurfactant causes an increase in the negative value of zeta potential. This adsorption changes the value of isoelectric point for serpentinite from pH = 4.5 to pH = 2.0 (Rodrigo et al., 2014).

4. Conclusions

In the present study, we have identified three potential bacterial strains from seawater associated with mangroves trees. All three bacterial strains are showed significant biosurfactant production capability. Growth conditions including pH and temperature are optimized and found that pH 7.0 and temperature 40 ºC are optimum for all three strains. FT-IR and GC-MS analysis confirm that extracted biosurfactant are lipopeptide in nature with higher emulsification activity. The Electrokinetic approach along with biosurfactant addition enhanced the biodegradation efficiency of bacterial strains. The biodegradation efficiency of crude oil by *B. subtilis* AS2, *B. licheniformis* AS3, and *B. velezensis* AS4 was about 88%, 92%, and 97% respectively. Addition of biosurfactant into the crude oil solution solubilizes them which make them more accessible by the bacterial strains. This integrated approach can be applied to remediate the crude oil contaminated soil with an eco-friendly manner.

CRediT authorship contribution statement

Arumugam Arul Prakash: Experimental work, Field collection, Writing - original draft. **Nataraj Srinivasa Prabhu:** Methodology, Field collection. **Punniyakotti Parthipan:** Validation, Writing - review & editing. **Mohamad S. AlSalhi:** Resources, Funding acquisition, Writing - review & editing. **Sandhanasamy Devanesan:** Validation, Formal analysis, Writing - review & editing. **Aruliah Rajasekar:** Project administration, Supervision, Validation, Writing - review & editing. **Muthusamy Govarthanan:** Writing - review & editing, Scientific discussion.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2020.124061.

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