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Screening For Potential Biosurfactant Producing Bacteria From Hydrocarbon–Degrading Isolates

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ABSTRACT
Many microorganisms are able to produce a wide range of amphipathic compounds are surface-active or biosurfactant. In the past decade, biosurfactants has attracted properties like specificity, low toxicity and relative ease of preparation. These properties for reducing dependence over environmentally harsh chemical detergents. This study aims to screen fifteen isolates for gasoline degradation and biosurfactant production**.** Different screening methods namely drop collapse, oil displacement test, hemolysis test, hydrophobicity; emulsification index and surface tension were used for their efficiency to potent biosurfactant production. All isolates were grown in mineral salt medium with 10% (v/v) of gasoline as carbon sources. Based on the obtained results three bacteria that were considered as efficient bacteria to gasoline degrading were identified from a previous study, however, we have shown that Pseudomonas *aeruginosa* 28 is more efficient than *Pseudomonas aeruginosa* HNYM41 and *Serratia marcescens* showing average gasoline degradation of 79.7%, 74.5% and 70.9% respectively but 98.4%, 94.6% and 97.2% for adhesion to hydrocarbon. In screening method six isolates showed positive results in drop collapse but the highest displace area for crude oil was given by *S. marcescens* and *P. aeruginosa* 28, at 9.43 mm and 9.42 mm. The selected isolate *S. marcescens* exhibited a high emulsifying activity of 29.2% compared the other species. The biosurfactant production reduced the surface tension of pure water from 69.6 mN/m to the 36.5 mN/m. For this reason among the fifteen isolates studied, *S. marcescens* was selected for gasoline degrading bacteria and biosurfactant producing. These surface-active increase the surface area of hydrophobic water-insoluble substrates and increase their bioavailability. The maximum biosurfactant production for these isolates could be considered as a potential biosurfactant producer at 0.6 g/L whiles the lower production by isolate *Bacillus licheniformis* at 0.18 g/L. The major objective of this study is to select potential isolate for gasoline degradation and biosurfactant production to using in environmental application.

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INTRODUCTION

Environmental pollution caused by petroleum and its derivatives, such as heavy oil, diesel fuel, gasoline, mineral oil, and engine oil has a growing impact on the important issues of ecological restoration [1]. Some biological technologies using natural or specialized microorganisms have been developed for the clean-up of oil-contaminated soils [2]. Biosurfactants produced by a variety of microorganisms are important biotechnology products for industrial applications because of the low toxicity, relative ease of preparation and widespread applicability to be used as emulsifiers, foaming agents and as detergents in petroleum [3]. It is used in environmental management in the petrochemical industry to enhance oil recovery of hydrocarbon remediation [2,4,5]. All biosurfactants are amphiphiles, which consist of two parts—a polar (hydrophilic) moiety and non-polar (hydrophobic) group [6]. These different polar hydrophilic head and hydrocarbon tail moieties decrease surface and interfacial tension by accumulating at the interface between immiscible fluids such as water and oil, or air and water these properties resulted in detergency, foaming and emulsifying [2,7].

Biosurfactants are structurally contains various group of molecules, that includes simple molecules (hydrophilic) which consist of mono-or oligosaccharides (rhamnose, mannose, glucose, galactose) polysaccharides, peptides or protein and high molecular weight (hydrophobic) moiety usually contains saturated, unsaturated and hydroxylase fatty acids [3,8,9,10]. Microorganisms producing biosurfactants assist to amplify the bioavailability of hydrocarbons by enhancing the contact between pollutant and the bacteria in the

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presence of the biosurfactant which helps by accelerated remediation of hydrocarbon contaminated sites [11]. Biosurfactants play an important role in the biodegradation or phytoremediation of organic contaminant such as crude oil, polycyclic aromatic hydrocarbons and polychlorinated biphenyls have received more attention in recent years [12]. Biosurfactants have high-molecular-weight showed a great potential in stabilizing emulsions between liquid hydrocarbons and water, thus increasing the surface area available for bacterial biodegradation, however they have been rarely tested as enhancers of hydrocarbon degradation in bioremediation [13].

Surfactants are used for soil washing due to their ability to assemble pollutants, amphiphilic compounds that reduce the free energy of the system by replacing the volume molecules of higher energy at an interface; they contain a hydrophobic portion with little affinity for the bulk medium and a hydrophilic group that is attracted to the bulk medium, surfactants use in industrially as adhesives and demulsifies the contaminants [12,14]. The capability of biodegradation by a biosurfactant is probably due to the increase of cell surface hydrophobicity that allows direct contact between the cell and the hydrocarbon droplets [15]. Biosurfactants play an important role in the biodegradation or phytoremediation of organic contaminant such as crude oil, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) have received more attention in recent years. In screening for microorganisms the most important surface-active properties evaluated with potential industrial application are surface tension reduction and emulsion forming and stabilizing capacity [16]. The main objective of this study was to screen microorganisms with elevated potential are capable of producing biosurfactant to improve biodegradation process of petroleum pollutants.

MATERIALS AND METHODS

Source and preparation of bacterial inoculums:

Total of 15 isolates collected from the microbe laboratory from Universiti kebangsaan Malaysia (UKM) as code mention in (Table 1). This isolates was collected and identified from previous students using biochemical and PCR technique that started from 2010 to 2011. Single bacterial isolate was grown in 40 mL of nutrient broth and incubated at 37°C in orbital shaker at 150 rpm, for 24 hours. The cells were separated by centrifugation (4000 rpm, 15 minutes, and the pellet was washed twice with the normal saline (pH 7.0). Then, the cells were resuspended in normal saline. Standardized inoculate was prepared by measuring the optical density at 550 nm using a UV- spectrophotometer. The experiment was done in triplicate.

Screening isolates for degrading gasoline:
Preparation of culture medium:

The mineral salts medium was prepared using distilled water as follows (g L^{-1}): 1.2 KH_2PO_4, 1.8 K_2HPO_4, 4.0 NH_4Cl, 0.2 $MgSO_4.7H_2O$, 0.01 $FeSO_4.7H_2O$ and 0.1 NaCl) and 0.1% of trace elements was added (g L^{-1}): 0.1 $MnSO_4.H_2O$, 0.025 $CuCl_2$, 0.025 $(NH_4)_6MO_7.O_{24}$, 0.025 CO $(NO_9)_2.6H_2O$, 0.025 ZnCl, 0.01 NH_4NO_3. The pH of the MSM medium was adjusted between 7.0-7.2 using 1 mol L^{-1} sodium hydroxide (NaOH) and hydrochloric acid (HCl), then the medium was autoclaved at 121^{o}C for 15 minutes [17].

Bacterial growth and colony forming unit (CFU):

The growth of bacteria was performed by using the mineral salt medium as describe above. Ten percent of standardized inoculums were inoculated into 100 mL of mineral salt medium (MSM) with 10% (v/v) of gasoline (pH 7.0) and incubated at 37 °C, 150 rpm for 5 days. Bacterial growth is determined by optical density measurement (OD) in (MSM), at wavelength 550 nm (OD_{550}) by using spectrophotometer device (Genesys 10 UV SPECTRONIC Thermo, USA). Growth is measured for each sampling was examined have the ability to survive in gasoline and its examined through colony-forming unit CFU/mL^{-1} count on the fifth day.

Degradation of Gasoline:

A total of 15 potential isolates were selected to quantify for the percentage of gasoline degradation. Ten percent of standardized inoculums were inoculated into 100 mL of mineral salt medium (MSM) with 10% (v/v) of gasoline (pH 7.0) and incubated at 37°C, 150 rpm for 5 days. The medium without the inoculation of bacteria was used as the control. The residual hydrocarbon was extracted from the culture medium with 100 mL of dichloromethane in 500 mL separator funnel. The solvent was then removed by evaporation using a rotary evaporator at 50°C. The residuals hydrocarbons were put into a 10 mL vial and then evaporated from 1 to 2 days in an overhead fume hood. The extract was then concentrated to 2 mL volume and analysed for TPH by GC–FID using a capillary column (Agilent Technologies, Model 7890A, GC system, U.K.**)** with an HP-5 5% phenyl methyl siloxane column (30 m x 0.32 mm i.d x 0.25 micron) with Helium as the carrier gas. The column temperature was programmed to stay at 50°C for 1 minute, and then ramp at 15°C per minute to 320°C for 10 minute**.** The percentage of TPH removal on each sampling bacteria was determined by using Equation

$$\%\ \text{Biodegradation} = \frac{TPH_0 - TPH_t}{TPH_0} \times 100$$

Where TPH_0= total petroleum hydrocarbon sampling on 0 hour
TPH_t= total petroleum hydrocarbon on each sampling after 5 days

Screening isolates for biosurfactant production:

The composition of mineral salts medium (MSM) used in this study was described above. Ten percent of standardized inoculums were inoculated into100 mL of mineral salt medium (MSM) with 10% (v/v) of gasoline as carbon sources (pH 7.0) and incubated at 37°C, 150 rpm for 5 days. The bacterial cultures were centrifuged at 8000 rpm for 15 minutes at 4°C and the culture suspension was screened for biosurfactant production by different methods.

Determination of the collective cell surface hydrophobicity (CSH):

Cell surface hydrophobicity was determined by bacterial adherence to the hydrocarbon as described by Rosenberg *et al.* [18]. Bacterial isolate were separated from culture medium after 5 days by centrifugation at 8000 rpm for 10 minutes and washed twice in normal saline. The cells were suspended in the same solution to an initial absorbance at 550 nm. Added 2ml cell suspension to 100 µl gasoline and vortexes for 3 minutes in test tubes and the aqueous phase were allowed to separate for 15 minutes. The OD was read from the aqueous phase at 550 nm. Hydrophobicity is expressed as the percentage of cell adherence to hydrocarbon calculated as follows:

Eq. (1): 100 *(1- OD of the aqueous phase / OD of the initial cell suspension).

Drop collapse test:

Qualitative drop collapse test was performed following the protocol of [19]. 2 µL of gasoline in the surface of glass plates and 5 µL of culture supernatant was added. After 1 minute observation the supernatant make the oil drop collapsed was indicated as positive results and that drops remain beaded were scored as negative result compared with distilled water as control.

Blood hemolysis test:

The isolates were screened by hemolytic activity on blood agar plates (peptone: 10 g/L; yeast extract: 3g/L; NaCl: 5g/L; sheep blood: 100 ml/L) hemolysis test is a primary method for screening microorganisms capable of producing biosurfactants, according to Liu *et al*. [19] 50 µL supernatant was added on blood agar incubated in 37°C for 48 - 72 hours. The bacterial colonies were then observed for the presence of clear zone around the colonies. This clear zone indicates the presence of biosurfactant producing organisms [14].

Oil displacement test:

The oil displacement test was used to measure the diameter of the clear zone by adding 50 mL of distilled water to a large petri dish (15 cm diameter). After that 20 µL gasoline was dropped onto the surface of the water followed by addition of 10 µL of cell culture supernatant. The diameter and the clear halo visualized under visible light were measured after 30 s. Each experiment was repeated three times to determine an average diameter of the clear zone [20,21].

Surface tension measurement:

Culture samples were centrifuged at 12,500 rpm for 15 minutes to remove cells and the resultant supernatant was submitted to surface activity measurements. Surface tension was measured by using a du Nouy ring-type tensiometer model KSV-sigma 703D (Finland) [8].

Emulsification index:

The emulsifying activity of biosurfactant capacity of the biosurfactant towards gasoline was done using a method by Cooper and Goldenberg [22]. A mixture of 4mL gasoline and 4 mL cell free extract obtained after the centrifugation of sample supernatant were taken in a test tube and homogenized by vortex for 2 minutes. The emulsion activity was investigated after 24 hours and the emulsification index (E24) was calculated by the total height of the emulsion by the total height of the aqueous layer and then multiplying by 100. The results were compared with 1% sodium dodecyl sulfate (SDS) as positive control.

Extraction of biosurfactants:

After screening for biosurfactant producing bacteria some isolates were selected for extraction of biosurfactants. Extraction as described by [14]. The culture was inoculated in 100 ml of MSM broth with 10% of gasoline for five days at 37^0C with shaking condition 150 rpm. After incubation the bacterial cells were

removed by centrifugation 8000 rpm, for 15 min at 4^0C. The supernatant was taken and the pH of the supernatant was adjusted to 2, using 1N HCl. Then supernatant was centrifugation 12000 rpm, for 15 min at 4^0C. Extracted with Chloroform and methanol (2:1 v/v). The solvents were removed by rotary evaporation and the resultant residue obtained was crude biosurfactant. Weight of the biosurfactant was expressed in terms of grams per liter (dry weight).

The dry weight of the biosurfactants was calculated by the following formula:

Dry weight of biosurfactants = (weight of the plate after drying with biosurfactant - weight of the empty plate)**.**

Results:

Bacterial growth:

Origins of the screened bacteria used in this study are listed in (Table 1). A total of 15 isolates were directly in culture medium MSM utilized10% (v/v) gasoline as growth substrates. Only three isolates (UKMA4, UKMA6 and UKMSM) showed similar response to 10% (v/v) gasoline concentration were having high growth rate in gasoline but reduced the growth rate for all isolates. In this test growth was expressed in CFU / mL^{-1} for 24 hours. Figure 1 show the 3 isolates rhizobacteria that can grow with 10% gasoline. The number of colonies enumerated in the plates was count on 10^7. The overall population means of isolates a range between (2.37, 2.03 and 1.99) CFU /$mL^{-1} \times 10^7$for isolates (UKMA4, UKMA6 and UKMSM) respectively. The bacterial growth was expressed of absorbance reading as optical density (OD) (Figure 2). OD range between 0.355 to 0.105 absorbance. Three isolates (UKMA4, UKMA6 and UKMSM that were dominant with count exceeding the range between (0.355 to 0.293), were selected. The results showed that three isolates could utilize a higher concentration of gasoline.

Degradation of gasoline:

Biodegradation of gasoline in MSM broth carried out on 15 isolated. Among 15 isolates three efficiently degrading isolates UKMA4, UKMA6 and UKMSM showed high degradation percentage of 79.9%, 74.5% and 70.9% respectively Figure 2 where all the three could excrete biosurfactant during the degradation of gasoline. Bacteria UKMA4 have the greatest degradation ability for gasoline compare with other isolates. These isolates grew well on medium containing gasoline, the degradation efficiencies of strain UKM A4 after 5 days. Strain UKMA4 had the greatest degradation ability for gasoline, compare with other isolates. But it had less degradation ability for gasoline the isolate UKM B7 with an efficiency of 17.05%. To degrade gasoline and other oil compounds, therefore in future these strains (UKMA4, UKMA6 and UKMSM) maybe have beat applying prospect in spot remediation of gas-station-leaking contaminated soil.

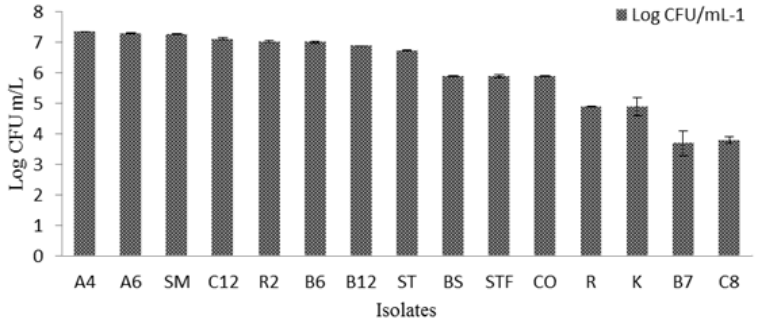

Fig. 1: Effect of gasoline on the growth of different isolates

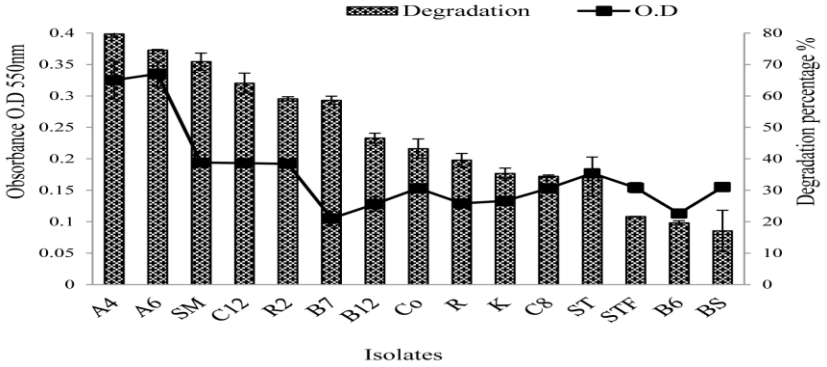

Fig. 2: The level of growth (measured as OD 550nm) and degraded gasoline for 15 isolates in MSM with 10% gasoline incubated at 37°C for 150 rpm after 5 days.

Screening isolates for biosurfactant production:

Results from various screening protocols to identify potential biosurfactant producers are listed in (Table 1). Fifteen bacterial isolates successfully used for screening biosurfactant activities by drop collapse test confirmed the production of biosurfactant by the bacterium and oil displacement test. Six isolates showed positive reaction for drop collapse test. The oil displacement tests in the present study of the crude biosurfactant have high surface activity and showed positive results for *S. marcescens.* These biosurfactant producers able to displace the oil at 9.43 mm in comparison to the commercial surfactant 1% SDS at 804 mm. Some isolates showed positive reaction for drop collapse on an oily plate. The drop collapse assay relies on the destabilization of liquid droplets by surfactants that correlate with surface and interfacial tension. The oil displacement tests in the present study indicated that (Figure 3). The halo ring around colonies on blood agar from culture supernatants by *S. marcescens* higher rate of hemolysis activity of the diameter 3.2 cm (Figure 4).

Cell surface hydrophobicity test:

The bacterial adhesion to hydrocarbons (MATH) assay was used to determine changes in cell surface hydrophobicity during growth. In this research the hydrophobicity range between 98.4% to 59.3% while result to *S. marcescens* high rate at 97.2%. This may facilitate cell adhesion and access to the substrate, as suggested by the subsequent maximal surfactant secretion.

Emulsification index and surface tension:

The highest emulsification index E24 value was observed in the crude biosurfactant from *S. marcescens* was found to be 29.15%. The emulsification indices shown in (Figure 5). Isolates in this study were eventually chosen for its ability to grow in MSM with gasoline as substrate and its capacity to decrease surface tension screening for the ability of biosurfactant production (Figure 6). A good surfactant can lower surface tension of water from 69.63 to 29.89 mN/m in 1% SDS. Cell-free supernatant from only five isolates (UKMSM, UKMSTF, UKMK, UKMC8 andUKMR2) exhibited reduced surface tension (Figure 6). The initial surface tension of the supernatant in culture medium at 55.49 mN/m reduction started rapidly by S. *marcescens* to 36.5 mN/m, the emulsification indices and surface tension of both are illustrated in (Figure 6).

Fig. 3: Zone formations by biosurfactants producing bacteria in oil displacement test

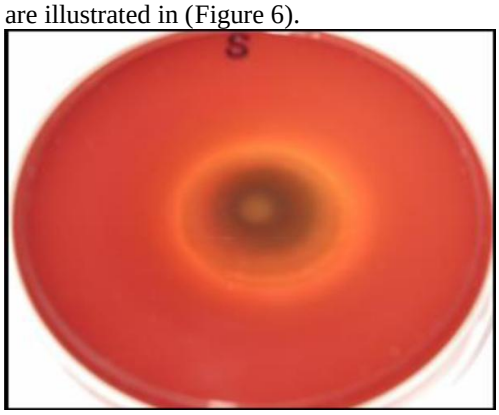

Fig. 4: Hemolytic activity on blood agar plated with UKMPSM isolate

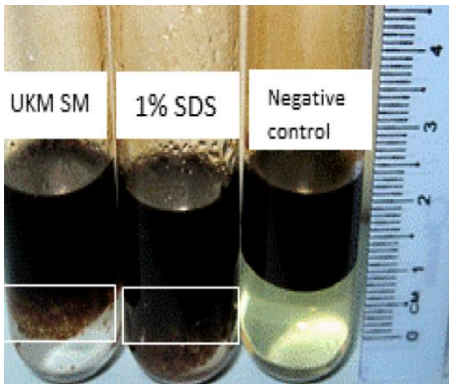

Fig. 5: The emulsion layer of UKMSM isolate mixed with crude oil at 24h.

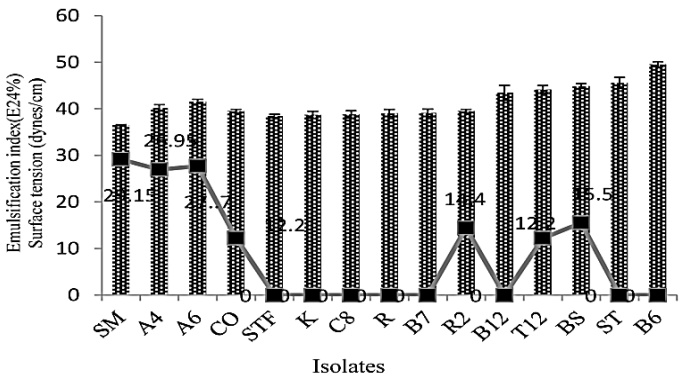

Fig. 6: Screening of biosurfactant producers by 15 isolates.

Extraction of biosurfactant:

Table 2 illustrated 8 isolates selected from the 15 isolates tested on the screening methods biosurfactant production to detect positive for potent biosurfactant production. It showed that the maximum production by *S. marcescens* was at 0.6 g/L all cultures but the lower production by isolate UKMPR was at 0.18 g/L.

Table 1: Screening results of biosurfactant producing bacteria

Sample code	Name of bacteria	Drop-Collaps test	Oil displacement area (mm)	Math assay (%)	Diameter of clearing zone(cm)
UKMA4	*Pseudomonas aeruginosa* 28	+	9.4±0.1	98.4±1.1	2.8±0.1
UKMA6	*Pseudomonas aeruginosa* HNYM41	+	7.8±0.1	94.6±1.4	2.9±0.6
UKMSM	*Serratia marcescens*	+	9.4±0.1	97.2±1.0	3.2±0.3
UKMC12	*Acintobacter spp*	-	7.8±0.2	91.2±0.7	1.8±0.3
UKMR2	*Rhodococcus spp*	-	7.8±0.1	96.8±0.4	1.7±0.2
UKM ST	*Stenotrophomonas maltophilia*	-	6.3±0.1	94.3±2.5	1±0.20
UKMBS	*Bacillus spp.*	-	7.8±0.2	73.7±0.2	1.8± 0.1
UKMSTF	*Staphylococcus aureus*	-	3.9±0.1	67.0±0.3	0.4±0.1
UKMCO	*Corynebacterium spp*	+	4.7±0.1	86.4±2.7	0.5±0.2
UKMC8	*Bacillus aquimaris*	-	4.7±0.1	59.3±0.9	0.2±0.2
UKMR	*Bacillus licheniformis*	-	3.2±0.1	85.9±2.8	0.3±0.1
UKMK	*Sphingomonas paucimobilis*	-	4.7±0.1	91.8±0.7	0.2±0.1
UKMB12	Bacillus *subtilis*	+	6.3±0.1	91.3±2.1	0.7±0.2
UKMB6	*Bacillus spp*	-	4.7±0.1	89.1±2.9	1.6±17
UKMB7	*Bacillus cereus*	+	6.2±0.1	64.1±1.7	0.0.05
Distal water		-	0	0	0
MSM+10% gasoline		-	0	0	0
1% SDS		+	804±0.1	0	2.1±0.1

Table 2: Biosurfactant production in g/L from different isolates

Isolates	Biosurfactant Production g/L
UKMSM	0.6±0.04
UKMA4	0.34±0.1
UKM6A	0.32±0.1
UKMCO	0.28±0.3
UKMC8	0.20±0.1
UKMSTF	0.22±0.1
UKMK	0.19±0.2
UKMR	0.18±0.3

Discussion:

Utilization of petrolum hydrocarbon and capability to produce biosurfactant by bacteria are interesting biotechnological applications as they can be used in different industrial processes [21]. The present study was initiated the isolates grow in MSM with 10% gasoline to enhanced the degradation especially in remediation process because the bioavailability and biodegradation of gasoline would be increased by biosurfactant and biosurfactant would play more roles in oil compound degradation [23]. According to the CFU results 8 isolates were chosen that can survive and tolerate with 10% gasoline the range of CFU /mL^{-1} from 2.35 to 1.05 (Figure 1). The growth rate of different isolates was observed for 5 days as shown in (Figure 2). Bacterial associated with the production of biosurfactants, we can conclude that the hydrocarbons metabolizing bacterium is able to secrete biosurfactants which enhance the hydrocarbon degradation further understanding of this mechanism will help and developing strategies for removing hydrocarbons from polluted areas [24]. Mehdi and Giti (2008) [25] demonstrate that bacteria has high hydrophobicity it can produce more biosurfactants and this enhances crude oil biodegradation. Biodegradation of gasoline by bacteria appears to be the natural process of the polluting hydrocarbon is used as an organic carbon source, causing transformed petroleum components to lower molecular compounds or into the other organic compounds such as biosurfactants [24].

In our study from 15 isolates three bacteria (UKMA4, UKMA6 and UKMSM) were the most efficient in degrading gasoline and produced potent biosurfactants. This results are in agreement with Si-jin *et al*., (2006) [23] investgated the biosurfactant excreting by three of strains *Pseudomonas* sp., *Flauobacteriurn sp*. and *Rhodococcus sp*. respectively may have great ability enhanced the degradation of gasoline. Six isolates showed positive reaction for drop collapse on an oily plate. The drop collapse assay relies on the destabilization of liquid droplets by biosurfactants this is correlates with surface and interfacial tension [26]. The oil displacement tests in the present study indicated that, the surface activity by measurement of a surfactant sample tested against oil is by addition of culture supernatant to spread and formed a wide clear zone on the oil-water surface [20,21] suggesting that it indicates potent surfactant. The results for oil displacement test by biosurfactants are shown in

Table 1 culture had the average area amounted 9.4 mm by two isolates *S. marcescens and P. aeruginosa* while the lowest amount was 3.2 mm by *Staphylococcus aureus.*

Colonies in blood agar with clear hemolytic or oil-dissolving circles were observed when the enriched culture broth was spread is widely used to screen for biosurfactant production. These organisms produced haemolytic activity as a sign for biosurfactant production due to the presence of transparent clearing zones observed in the blood agar plates [27]. The halo ring around colonies on blood agar by *S. marcescens* higher rate of hemolysis activity of the diameter causing domains or pores within the erythrocyte membrane and the biosurfactants interact strongly with cellular membranes while the exotoxins cause lysis of the red blood cells because biosurfactant have amphiphilic in nature can partition into the phospholipid membrane of this cell [4]. The similarly result was correlates with the studies of observed the culture producing hemolysis was able to produce biosurfactants [14]. Who used blood hemolysis test for screening biosurfactant producing organisms. Nishanthi *et al*. [26] revealed Serratia *spp.* having higher rate of hemolysis activity of the diameter 11mm. Emulsification index is one of the creteria to support to choice of potential biosurfactant. Emulsification execute when the surface tension reduction and reduced interface between oil and water provided excellent properties in terms of reduction of surface tension [4]. The biosurfactant possessed high surface activity that could lower the surface tension. The surface tensions of the controls were as follows: deionized water 69 mN/m, the isolates able to lower the surface tension of MSM blank when grown on gasoline as the sole source of carbon can produce higher biosurfactants [28].

In this study result obtain the surfactant reduce maximum surface tention by *S. marcescens*. These results confirm with the activity of the bacteria depends on the hydrophobic substrate in the aqueous phase and secreted biosurfactant [29]. This investigation suggested that increases in the hydrophobicity of the cell surface during the growth on hydrocarbons and enables bacteria to degrade the components of gasoline. Surfactants increase in the cell surface hydrophobicity by the attachment of cell to hydrocarbons thereby enhances the degradation during the biodegradation process [2,24]. Results showed that the high hydrophobicity by three bacteria (UKMA4, UKMSM and UKMA6 were 98.4%, 97.2% and 94.6% respectively. In our study, the three selected isolates produced biosurfactants when incubated with gasoline, suggesting that the addition of gasoline would increase bacterial biosurfactant production. Zhang *et al*. [28] showed the used of organic compound as carbon sources to enhancement production. Biosurfactant production was tested from eight out of fifteen isolates to detect positive for potent biosurfactant production. All the eight cultures showed maximum production by *S. marcescens.* These results is in agreement with Nishanthi *et al*. [26], where the two isolates *Pseudomonas sp* and *Serratia sp* showed maximum biosurfactant at 0.8 g/l and 0.6 g/l respectively.

Conclusions:

A total of 15 isolates were screened for gasoline degradation and biosurfactant production which has a promising use in environmental application. Three isolates (UKMA4, UKMA6 and UKMSM) showed higher degradation while only one bacterial isolate are capable of producing higher potent biosurfactant as compare to other.

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