Abstract

A total of ten bacterial isolates were screened for their biodegradation, metabolic versatility and biosurfactants production using various organic pollutants. The biosurfactants production ability was mainly assessed by oil spread test (OST) and/or emulsification assay (EA). Although initial biosurfactants screening was conducted using paraffin oil, the application of vegetable oils, particularly coconut oil, was always accompanied with the highest yield of biosurfactants production. Biochemical and molecular identification of the ten isolates revealed that they belong to three genera: Klebsiella (6), Pseudomonas (3) and Citrobacter (1). Interestingly, four isolates (M2H2 1, M2H2 3, M2H2 8 and M2H2 14), showed the highest biosurfactants production and therefore were further assessed using mixed carbon source (coconut oil in combination with one organic pollutant (phenol or cyclohexanol)). The addition of the coconut oil was essential for increased production of biosurfactant, while the use of organic pollutant as a sole carbon source was always accompanied with lower productivity. Isolates (M2H2 1 and M2H2 14), showed the highest phenol biodegradation capacities (the most toxic pollutant), and were tested for the dual effect of biodegradation combined with biosurfactant production. Isolates M2H2 1 and M2H2 14 tolerated phenol concentrations up to 1500 and 1300 mg/l, respectively, with no significant effect on biosurfactant activity. Adopting the induction regimen increased the phenol removal percentage from 2% to 66% and from 10% to 35% with isolates M2H2 1 and M2H2 14, respectively.

Keywords: Emulsification; Oil; Bioremediation; Phenol

Introduction

Bioremediation provides a high potential and cost efficient tool for the treatment of toxic pollutants in different environments [1,2], however, it still facing several limitations [3]. These limitations include the response to fluctuation in the concentration and/or the content of pollutants load. For instance, increasing the concentration and/or the toxicity of the polluting compounds may inhibit the growth of the biodegrading species [4]. Consequently, this would lead to the failure of the whole bioremediation process. In addition, the hydrophobic property of many of these compounds severely limits the mass transfer during the biological degradation [5] leading to poor availability of these compounds to the microbial cells. In this sense, the addition of surfactants and/or biosurfactants has been regarded as a promising approach to increase the bioavailability as well as the biodegradation efficiency [6].

Biosurfactants have a direct effect through increasing the solubility and dispersion of the hydrocarbon, hence increasing its availability for the microbial cells. Besides, they may change the affinity between microbial cells and hydrocarbons by inducing rises in cell surface hydrophobicity [7,8], thus improving the biodegradation efficiency. Interestingly, biosurfactants could have stimulating effects on enzyme activities or production by microorganisms through their effect in aiding enzyme release [9,10] and increasing the enzyme stability through prevention of the enzyme denaturation during hydrolysis by desorbing them from substrate [11]. Hence, the use of microbial strains that would have dual effect (degradation of the pollutants and production of the biosurfactants) would have several advantages [12].

In comparison with synthetic surfactants, biosurfactants have many features which have made them gain an increased attention. Biosurfactants have advantages that include higher biodegradability, lower toxicity, lower cost, selectivity and specific activity at extreme temperatures, pH and salinity [13]. On the other hand, synthetic surfactants currently used are toxic and are hardly degraded by microorganisms causing damage to the environment [14].

In the present study, we aimed at screening, isolating and characterizing bacterial isolates with relevant dual biodegradation and biosurfactants production ability. The isolates were tested for biosurfactants production, biodegradation ability and for their possible dual property. The effect of using different carbon sources was investigated as well as their toxic effect on the most relevant isolates.

Materials and Methods

Unless otherwise specified, all experiments were conducted under aseptic conditions and in triplicate. The data values shown represent the mean ± standard error of conducted replicates.

Enrichment, isolation and maintenance of degrading bacterial isolates

Screening, isolation and maintenance of all microbial cultures were conducted using a mineral salt medium (MSM) with composition as previously described [15]. Whenever necessary, the MSM was enriched with required concentration of the selected substrate (Table 1) and/or solidified with 2% (w/v) Nobel agar (Oxoid, USA). Bacterial strains were...
isolated from several soil samples, collected from different locations in Giza and Cairo. Isolation was conducted as previously described [15] with incubation conditions of 28 ± 2°C and agitation at 180 rpm.

Morphological, physiological and molecular identification

Morphological characterization and motility tests were done using light microscopy (Olympus, USA). Gram stain reaction was done using Difco Gram stain set according to the standard protocol and as previously described [16]. Biochemical characterization was done using API 20 NE or 20 E kit systems (bioMérieux, France) according to the manufacturer’s instructions. The presence of oxidase was determined using a test strip (Microbiology Bactident Oxidase, Merck, Germany). Catalase activity was evaluated by transferring a loop of bacterial cells onto a microscope slide and adding a drop of 3% hydrogen peroxide solution [15].

Molecular identification was carried out using partial 16S rRNA sequence analysis [15,17] using two universal primers 28F (forward primer) 5′AGAGTTTGATCCTGGCTCAG-3′ (positions 8-28) and 1512R (reverse primer) 5′ACGGCTACCTTGTTACGACT-3’ (positions 1512-1493), (E.coli. numbering). The GenBank database (NCBI, USA) was then used to search for 16S rRNA sequence similarities.

Biodegradation and metabolic versatility

For each of the ten isolates, an aliquot of 5 ml of the bacterial suspension (106-107 CFU ml−1) was used to inoculate flasks containing 100 ml MSM supplemented with increasing pollutant concentrations (Table 1). The flasks were incubated for 3 and up to 28 days at 28 ± 2°C in an incubator shaker at 180 rpm. Samples were periodically withdrawn and tested for growth and/or pollutant removal. A significant increase in the optical density at 600 nm was considered as positive growth [15]. Negative controls were conducted by using MSM with the pollutants and without inoculation or MSM inoculated with the isolate without organic carbon source.

**Screening of biosurfactant production ability of biodegrading isolates**

Biodegrading bacterial isolates were preliminarily screened for biosurfactant production using paraffin oil as substrate. Initially, the inoculum was prepared as previously described [15]. Aliquots of 100 ml MSM supplied with required concentration of substrate were placed in 250 ml erlenmeyer flasks and were inoculated by 5% (v/v) of each bacterial isolate. Flasks were incubated in incubator shaker at 180 rpm and 35 ± 2°C for 6 days and samples were withdrawn at the last day for analysis. Different oils (olive oil, castor oil, bitter almond oil and coconut oil), organic solvents (hexane, benzene and xylene) and organic pollutants (cyclohexanol) were used in order to evaluate their effects on biosurfactant productivity. In all cases, the collected samples were centrifuged at 4500 g for 30 min prior to analysis.

**Investigation of the dual effect "biodegradation coupled with biosurfactant production"**

The most relevant four isolates were selected to study the dual effect (coupled biodegradation and biosurfactants production), using the following combinations in (% w/v): coconut oil (2) + phenol (0.01), coconut oil (2) + cyclohexanol (0.025), phenol (0.01) and cyclohexanol (0.025). The control was 2% (w/v) of coconut oil. The isolates showing the highest recorded biosurfactant productivity and biodegradation capacities of phenol were further selected to investigate the dual effect; in the presence of increasing concentrations (100-1700 mg l−1) of phenol.

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**Table 1:** The recorded biodegradation ability and metabolic versatility of the isolates against different organic pollutants and/or organic solvents.

| Isolate  | Hexane 100 | Decane 100 | Dodecane 100 | Hexadecane 100 | Benzene 100 | Toluene 100 | Xylene 100 | Pyridine 100 | Phenol 200 | Cresol 100 | Salicylate 100 | Naphthalene 50 | Cyclohexanol 1000 |
|----------|------------|------------|--------------|----------------|-------------|-------------|------------|-------------|------------|------------|----------------|-----------------|------------------|
| Conc. (mg l⁻¹) | (+) means growth, (−) means no growth | (+) means growth, (−) means no growth | (+) means growth, (−) means no growth | (+) means growth, (−) means no growth | (+) means growth, (−) means no growth | (+) means growth, (−) means no growth | (+) means growth, (−) means no growth | (+) means growth, (−) means no growth | (+) means growth, (−) means no growth | (+) means growth, (−) means no growth | (+) means growth, (−) means no growth | (+) means growth, (−) means no growth | (+) means growth, (−) means no growth |
| Hexane   | 100        | +          | +            | −              | −           | +           | −          | +           | +          | +          | +              | −                | +                |
| Decane   | 100        | +          | −            | −              | −           | +           | −          | +           | +          | +          | +              | −                | +                |
| Dodecane | 100        | −          | −            | −              | −           | −           | −          | −           | −          | −          | −              | −                | −                |
| Hexadecane | 100       | +          | +            | +              | +           | +           | −          | −           | −          | −          | −              | −                | +                |
| Benzene  | 100        | +          | +            | +              | +           | +           | +          | +           | +          | +          | +              | −                | +                |
| Toluene  | 100        | −          | −            | −              | −           | −           | −          | −           | +          | +          | +              | −                | +                |
| Xylene   | 100        | −          | +            | +              | +           | −           | +          | −           | +          | +          | +              | −                | +                |
| Pyridine | 100        | +          | −            | +              | −           | +           | +          | +           | +          | +          | +              | −                | +                |
| Phenol   | 200        | +          | +            | +              | +           | −           | +          | +           | −          | +          | +              | −                | +                |
| Cresol   | 100        | +          | +            | +              | +           | +           | +          | +           | +          | +          | +              | −                | +                |
| Salicylate | 100       | +          | +            | +              | +           | −           | −          | +           | +          | +          | +              | −                | +                |
| Naphthalene | 50         | −          | −            | −              | −           | −           | −          | −           | −          | −          | +              | −                | +                |
| Cyclohexanol | 1000     | +          | +            | +              | +           | +           | +          | +           | +          | +          | +              | −                | +                |

(*) The maximum tested concentration.
as a model of organic pollutant. Samples of 5 ml were withdrawn for analysis at regular time intervals. Whenever necessary, an induction regimen (i.e. initial addition of low concentration of the pollutant) was conducted using 500 mg l\(^{-1}\) of phenol to establish enough biomass prior to the addition of more phenol and coconut oil.

**Analysis**

**Oil spreading test (OST):** A total of 50 ml of distilled water were added to a Petri dish (150 mm diameter) followed by the addition of 20 μl of oil (castor oil) on the water surface to form a thin oil layer. An amount of 10 μl of culture supernatant was gently placed onto the oil layer. The diameter of the clear zone was measured and recorded. In preliminary screening, test results with zone diameters greater than 0.5 cm were classified as positive [1].

**Emulsification assay:** An aliquot of 3 ml of the supernatant was vortexed with 0.5 ml castor oil for 2 min. The mixture was left undisturbed for 1 h at 28 ± 3°C to separate aqueous and oil phases. The aqueous phase was collected and measured at 400 nm using spectrophotometer (T80 UV/Vis spectrophotometer, USA). The emulsification units (EU) were calculated according to the following equation [18].

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\text{EU ml}^{-1} = \text{absorbance at 400 nm x dilution factor/0.01}
\]

**Blue agar plates method (CTAB agar plate method):** The CTAB agar plate method was used as a qualitative assay for the detection of anionic surfactants. The blue agar plates were prepared by adding 0.2 g cetyltrimethylammonium bromide (CTAB) and 0.005 g methylene blue (MB), 15 g agar and 20 g glycerol as a substrate to 1 l of the MSM [19]. Wells were cut in the agar plates [20], where 10 μl of the inoculum were added. The plates were incubated for 48 h at 37°C and then stored in the refrigerator for at least 24 h. Formation of colonies surrounded by dark blue halos indicated the production of anionic surfactants. Examination using UV transilluminator was used to light the plates for easier detection [21].

**Phenol analysis:** Analysis of phenol was conducted by HPLC-UV (Schimadzu 10A VP, USA), equipped with a Supelco LC-18 column with a detection limit of 1 mg l\(^{-1}\) as previously described [15].

**Statistical analysis:** The statistical analysis and graphical presentation of data was done using graphpad prism’ software (version 5.01).

**Results**

**Morphological, physiological and molecular identification**

Ten bacterial strains were isolated. Preliminary examination showed that they were all rod shaped bacteria with Gram negative reaction. Further identification of the isolates showed that all had positive catalase reaction. Only three isolates (M2H2 1, M2H2 14 and M2H2 15) were motile and had positive oxidase reaction (Table 3). Isolates M2H2 4, M2H2 7, M2H2 8, M2H2 10, M2H2 16 and M2H2 18 were identified biochemically (using API 20E kit system) and molecularly and were shown to be members of *Klebsiella* spp. Isolate M2H2 3 was classified molecularly as a member of *Citrobacter*

| Substrates          | Paraffin Oil 2% w/v | Olive Oil 2% w/v | Castor Oil 2% w/v | Bitter Almond Oil 2% w/v | Coconut Oil 2% w/v | Cyclohexanol 0.025% w/v |
|---------------------|---------------------|------------------|-------------------|--------------------------|--------------------|-------------------------|
| Isolates            | Test                |                  |                   |                          |                    |                         |
| M2H2 1              | OST (cm)            | 0.6 ± 0          | 8.9 ± 0.9         | 3.2 ± 0.7                | 4.6 ± 0.5          | 3.1 ± 0.1               | 1.8 ± 0.3               |
|                     | EA (EU ml\(^{-1}\))| 10 ± 2           | 67 ± 10           | 169 ± 16                 | 61 ± 2              | 75 ± 6                  | 10 ± 1                  |
| M2H2 3              | OST (cm)            | 1.9 ± 0.3        | 4.5 ± 0.2         | 4.4 ± 0.7                | 3.1 ± 0.2          | 4.8 ± 1.1               | 0                      |
|                     | EA (EU ml\(^{-1}\))| 39 ± 8           | 94 ± 9            | 86 ± 7                   | 47 ± 4              | 265 ± 5                | 34 ± 1                  |
| M2H2 4              | OST (cm)            | 7.3 ± 0.6        | 5.5 ± 0.8         | 5.8 ± 0.5                | 3.6 ± 0.7          | 8.2 ± 0                 | 0                      |
|                     | EA (EU ml\(^{-1}\))| 54 ± 10          | 43 ± 6            | 68 ± 0.3                 | 31 ± 4              | 189 ± 7                | 42 ± 3                  |
| M2H2 7              | OST (cm)            | 5.6 ± 0.8        | 3.7 ± 0           | 4 ± 0.4                  | 4.8 ± 0.5          | 5.1 ± 0.8               | 0                      |
|                     | EA (EU ml\(^{-1}\))| 30 ± 4           | 44 ± 7            | 90 ± 17                  | 12 ± 0              | 410 ± 4                | 15 ± 2                  |
| M2H2 8              | OST (cm)            | 4.5 ± 0.9        | 6 ± 1.2           | 5.7 ± 0.8                | 5.8 ± 0.1          | 7.6 ± 0.2               | 0                      |
|                     | EA (EU ml\(^{-1}\))| 0.53 ± 0         | 66 ± 9            | 51 ± 5                   | 44 ± 2              | 169 ± 16                | 21 ± 2                  |
| M2H2 10             | OST (cm)            | 3.7 ± 0.5        | 7.2 ± 0.4         | 6 ± 0.6                  | 8.2 ± 1.1          | 7.7 ± 0.9               | 0                      |
|                     | EA (EU ml\(^{-1}\))| 2 ± 0            | 39 ± 1            | 48 ± 7                   | 29 ± 7             | 92 ± 3                  | 38 ± 5                  |
| M2H2 14             | OST (cm)            | 8.9 ± 8          | 11.3 ± 0.3        | 9.3 ± 0.4                | 9.8 ± 0.5          | 7 ± 1                   | 0                      |
|                     | EA (EU ml\(^{-1}\))| 13 ± 1           | 78 ± 8            | 24 ± 3                   | 338 ± 53           | 31 ± 4                  | 33 ± 4                  |
| M2H2 15             | OST (cm)            | 2.4 ± 0          | 7.6 ± 0.2         | 5.8 ± 0.7                | 9.8 ± 0.1          | 6.5 ± 1.1               | 0                      |
|                     | EA (EU ml\(^{-1}\))| 21 ± 3           | 62 ± 5            | 38 ± 4                   | 356 ± 33           | 21 ± 0.3                | 17 ± 3                  |
| M2H2 16             | OST (cm)            | 0                | 7.1 ± 0.7         | 6.4 ± 0.9                | 2.8 ± 0            | 6.7 ± 0.4               | 0                      |
|                     | EA (EU ml\(^{-1}\))| 23 ± 2           | 58 ± 9            | 38 ± 3                   | 52 ± 6             | 16 ± 1                  | 13 ± 3                  |
| M2H2 18             | OST (cm)            | 0                | 8.5 ± 0.3         | 7.1 ± 0.1                | 5.9 ± 1.3          | 9.6 ± 1.2               | 0                      |
|                     | EA (EU ml\(^{-1}\))| 2 ± 0            | 93 ± 14           | 33 ± 6                   | 42 ± 4             | 17 ± 4                  | 17 ± 3                  |

**Table 2:** The recorded biosurfactant activity according to oil spread test (OST) and emulsification assay (EA) using different substrates. Relevant isolates were incubated for 6 days at 35 ± 2°C.
biosurfactants were anionic surfactants. Haloes in the CTAB agar plates (Figure 1) indicating that the produced activities were recorded when vegetable oils were used and especially, different substrates and isolates (Table 2). The highest biosurfactant ANOVA revealed a significant difference in biosurfactant activity with when using any of the organic solvents (hexane, benzene or xylene) as organic pollutant (Table 2). No biosurfactant activity was observed different types of substrates; vegetable oils, organic solvents and Eventually, the biosurfactants production was investigated using paraffin oil (mineral oil) as the sole substrate. Screening of biosurfactant production ability of biodegrading Pseudomonas spp. (Table 3).

Biodegradation and metabolic versatility

The biodegradation versatility of the ten isolates was investigated against aliphatic hydrocarbons (hexane, decane, dodecane and hexadecane), aromatic hydrocarbons (Benzene, toluene, xylene, pyridine, phenol, cresol, salicylate and naphthalene) and cyclic hydrocarbons (cyclohexanol). All isolates were able to tolerate and metabolize hexadecane, benzene, cyclohexanol and cresol (Table 1). However, none of the isolates was able to grow on either dodecane or naphthalene (Table 1).

Screening of biosurfactant production ability of biodegrading isolates

Preliminary screening of the isolates for biosurfactant production was carried out using paraffin oil (mineral oil) as the sole substrate. Eventually, the biosurfactants production was investigated using different types of substrates; vegetable oils, organic solvents and organic pollutant (Table 2). No biosurfactant activity was observed when using any of the organic solvents (hexane, benzene or xylene) as sole carbon source (data not shown). Statistical analysis using two ways ANOVA revealed a significant difference in biosurfactant activity with different substrates and isolates (Table 2). The highest biosurfactant activities were recorded when vegetable oils were used and especially, Coconut oil (Table 2) and therefore it was selected as a substrate model for further experimental work. All isolates showed dark blue haloes in the CTAB agar plates (Figure 1) indicating that the produced biosurfactants were anionic surfactants.

Investigation of the dual effect "biodegradation coupled with biosurfactant production"

Four isolates (M2H2 1, M2H2 3, M2H2 8 and M2H2 14) were selected for testing the biosurfactants production ability in the presence of an organic pollutant as sole substrate and in combination with coconut oil. Addition of any of the tested organic pollutants had no significant effect on the biosurfactants production. Interestingly, biosurfactant activity was significantly reduced (by more than 70%) when either phenol or cyclohexanol were used as sole carbon source (Figure 2). Among these four isolates, two isolates (M2H2 1 and M2H2 14) were selected for further studies due to their recorded highest biodegradation capacity.

The dual effect (biosurfactant production and biodegradation)

Table 3: Summary of morphological, biochemical and molecular identification of the isolated bacterial strains (N/A means not applied).

| Isolate | M2H2 1 | M2H2 3 | M2H2 4 | M2H2 7 | M2H2 8 | M2H2 10 | M2H2 14 | M2H2 15 | M2H2 16 | M2H2 18 |
|---------|-------|-------|-------|-------|-------|--------|--------|--------|--------|--------|
| Test    |       |       |       |       |       |        |        |        |        |        |
| Motility| +     | -     | -     | -     | -     | +      | +      | -      | -      | -      |
| Oxidase | +     | -     | -     | -     | -     | +      | +      | -      | -      | -      |
| API 20E | N/A   | N/A   | N/A   | N/A   | N/A   | Pseudomonas | Burkholderia spp. | N/A | N/A |
| API 20NE| N/A   | Klebsiella spp. | N/A   | N/A   | Klebsiella spp. | Pseudomonas | Burkholderia spp. | N/A | N/A |

![Figure 1: TBlue agar plates (CTAB agar plates) showing dark blue haloes indicating the production of anionic surfactants. Ten micro liters of the inoculum were added into each well, plates were incubated for 48 h at 37°C and then stored in the refrigerator for 24 h. Dark blue haloes were then detected using normal light condition (A) and UV transilluminator (B).](image)

![Figure 2: The recorded biosurfactant activity by the most relevant four isolates (M2H2 1 and M2H2 14) were selected for further studies due to their recorded highest biodegradation capacity. The dual effect (biosurfactant production and biodegradation)](image)
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Discussion

Molecular identification of the ten isolates revealed that they belong to three genera; Klebsiella (6), Pseudomonas (3) and Citrobacter (1). These three genera, in particular, possess interesting potential in term of both biodegradation and biosurfactants production [22-27]. For instance, members of Pseudomonas genus are well recognized for their capability of both degrading aromatic compounds especially phenol [25,27] and biosurfactant production mainly rhamnolipids [24,26]. As for Klebsiella genus, recent studies have reported the isolation of several Klebsiella species with diverse biodegradation ability [23,28]. Other studies were likewise concerned with characterization of the biosurfactant produced [22,29].

In this study, no biosurfactant activity was observed with the organic solvents used and the lowest biosurfactant productivity was observed when organic pollutants were used as sole carbon source. On the other hand, the use of vegetable oils (either as sole carbon source or mixed with organic pollutant) was always accompanied with higher production of biosurfactants. Several studies have reported similar patterns [30,31], which could be attributed to the high organic and nutrient content of the vegetable oils [32]. Concurrently, this may be also according to the concentration of the used substrate which in turn affects stoichiometric requirement for high productivity [33].

Interestingly, although the toxicity of the pollutants is among the effects that should be taken in consideration [27], the addition of the selected pollutants had no significant effect on the biosurfactants production. Consequently, the relevance of using high tolerant and biodegrading isolates should be explored. Based on the results obtained, coconut oil was selected as a substrate model for further experimental work. This is due to the high biosurfactant activity recorded when used as substrate in addition to the ease of the downstream processes as this oil tends to solidify as separate layer at room temperature.

Biodegradation combined with biosurfactant production was investigated in the presence of a model of organic pollutant (phenol). Isolates tolerated high phenol concentrations in the presence of oil with no significant effect on biosurfactant productivity. However, the phenol biodegradation property was significantly diminished. Biodegradation was further studied using coconut oil 2% w/v with adding increasing concentrations of phenol (100-1700 mg l⁻¹). The two isolates M2H2 1 and M2H2 14 retained their biosurfactant productivity (Figures 3 and 4) - no significant reduction - when increasing concentrations of phenol were added up to 1500 and 1300 mg l⁻¹, respectively. Nevertheless, phenol biodegradation was almost completely inhibited by both isolates (Data not shown).

Consequently, an induction procedure was adopted where 500 mg l⁻¹ of phenol was used to establish enough biomass prior the addition of additional 500 mg l⁻¹ phenol and coconut oil 2% w/v. Statistical analysis using paired t-test showed that for isolate M2H2 1, there was no significant difference in biosurfactant activity either with or without induction. However, phenol removal percentage differed significantly from 2% to 66% when using the induction regimen (Figure 5). A similar pattern was observed with isolate M2H2 14, where the phenol biodegradation was enhanced by the induction, however, statistical analysis using paired t-test showed insignificant difference with and without induction (Figure 5).

Figure 3: The recorded biosurfactant activity by isolate M2H2 1 using coconut oil 2% w/v with increasing concentrations of phenol (100-1700 mg l⁻¹). The biosurfactants activity was expressed as (zone diameter) based on the oil spread test (A) and Emulsification unit ml⁻¹ based on the emulsification assay (B).

Figure 4: The recorded biosurfactant activity by isolate M2H2 14 using coconut oil 2% w/v with increasing concentrations of phenol (100-1300 mg l⁻¹). The isolate was incubated for 6 days at 35 ± 2°C and agitation rate 180 rpm. The biosurfactants activity was expressed as (zone diameter) based on the oil spread test (A) and Emulsification unit ml⁻¹ based on the emulsification assay (B).
With induction then the oil and more 500 mg l\(^{-1}\) phenol were further added. Indeed, the observation was confirmed when an induction regimen was performed biodegradation when the coconut oil was added, where the lack of activities. This might explain the recorded deterioration of the phenol during the formation of enough biomass and the essential enzymatic and adequate fast mass transfer [34,35], especially at the initial stages of phenol is mainly aerobic and required sufficient oxygen concentration and increasing concentrations of organic pollutant (phenol) had no effect on biosurfactant production by these strains. Two isolates had potential dual effect; pollutants biodegradation and biosurfactants production. The present study provides a promising strategy and candidates for integrated and effective bioremediation purposes.

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Figure 5: Comparing biosurfactant activity and phenol removal percent recorded by isolate M2H2 1 (A & B) and isolate M2H2 14 (C & D) with and without induction regimen. Isolates were grown at 35 ± 2°C with agitation (180 rpm) for 6 days on MSM media supplied with coconut oil (2% w/v) together with phenol (500 mg l\(^{-1}\)). Induction regimen was conducted using 500 mg l\(^{-1}\) of phenol to establish enough biomass prior to the addition of additional phenol (500 mg l\(^{-1}\)) and coconut oil (2% w/v). *: indicates significant difference at P-value < 0.05 (paired t test).

Conclusion

A total of ten biodegrading bacterial isolates with relevant biosurfactant production ability were characterized. The presence of of phenol is mainly aerobic and required sufficient oxygen concentration and adequate fast mass transfer [34,35], especially at the initial stages during the formation of enough biomass and the essential enzymatic activities. This might explain the recorded deterioration of the phenol biodegradation when the coconut oil was added, where the lack of oxygen could have a limiting effect on phenol biodegradation [34]. This observation was confirmed when an induction regimen was performed where the biomass was allowed initially to degrade 500 mg l\(^{-1}\) of phenol then the oil and more 500 mg l\(^{-1}\) phenol were further added. Indeed, the initial induction allowed the building of enough biomass and induced the necessary enzymatic activities [36].
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