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TESTING EFFICIENCY OF NANOPARTICLES SUPPORTED WITH BIO-SURFACTANT EXTRACTED FROM *BACILLUS* SP. ISOLATED OF OIL-POLLUTED SOIL IN TREATING ENGINE OIL WASTES

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Abstract

Bio-surfactants are surface-active metabolites produced by microorganisms. The applications of these biological compounds in the field of enhanced oil recovery and bioremediation proved effective, consists of characteristically organic compounds containing both hydrophobic groups (their tails) and hydrophilic groups (their heads. A bio-surfactant extracted from *Bacillus* spp. cultured on the MSM media with (1% Vegetable oil + 1% Glucose) and MSM with (2% Vegetable oil), as carbon source, were better than MSM media containing (2% Glucose), after incubation for 7 days, at 30ºC and stirred at 150 rpm, with a decrease in surface tension of water from 72 to (32.50, 32.92, 56.42) mN/m, respectively. Result showed that *Bacillus* bio-surfactants produced in a low-cost medium formulated with vegetable oil, glucose, and distilled water was employed to stabilize silver nanoparticles in the liquid phase, and treated engine oil waste by mixing with (Bio-surfactant) and (Silver nanoparticle supplemented with bio-surfactant), then measured by GC chromatography after incubated for 48hr at room temperature was (13.60 and 96.69) %, respectively.

***Keywords*:** *Bacillus*, Oily polluted soil, Bio-surfactant, Bio-surfactant extraction, Silver nanoparticles.

Introduction

A surfactant is a surface-active composite consisting of hydrophobic and hydrophilic molecules, furthermore, can combine two immiscible composites, similar water, and oil within surface tension decreasing (Desai and Banat, 1997; Mulligan, 2005). Microbial excretion that displays distinct surface and emulsifying vitalities are identified as bio-surfactants. Both have principally utilized during ecological applications because of their variety, environment-friendly nature, agreeably to large scale product including selectivity, in spite of probable and their natural origin (Anila and Ayesha, 2016). So, bio-surfactants decrease the surface and interfacial tension in both aquatic solutions and hydrocarbon mixes, moreover produce micelles and micro emulsions among those phases (Letizia *et al.,* 2015). It is the common essential substances applied in different industries, such as emulsifiers, corrosion hindrance, foaming, detergent, conditioner hair, and improvement of oil recovery (Mulligan, 2005; De Oliveira *et al.,* 2013). Bio-surfactant as a typical surfactant formed via microorganisms from various substrates becomes attracted more attention to industries (Chen *et al.,* 2015).

Nanomaterial may ensure liquids to environmental defiance into these regions of solar energy transformation, stimulated, remedy, and water treating. The aforementioned increasing request requirement is escorted within "green" synthesis methods (Nafeesa *et al.,* 2017). A report by Priyadarshini et al. (2016), evaluating that bio-surfactants could operate as limiting and stability factors in the synthesis of nanoparticles. Nanoparticles, as proposed by Plaza *et al*. (2014), are usually synthesized by physiochemical techniques including the use of great force, heat, plus energy, whereas, utilizing analytic methods are usually correlated besides establishing of poisonous results. So, employing the biological substances as bio-surfactants has achieved important as construction and stabilization of nanoparticles for the latest decade (Kasture *et al.,* 2008; Kumar *et al.,* 2010).

This work aimed to: *Isolate bacteria from soil polluted with engine oil wastes, possess the ability to produce bio-surfactant. *Synthesizing silver nanoparticles supported with bio-surfactant. *Testing efficiency of nanoparticles supported with bio-surfactant in treating engine oil wastes.

Materials and Methods

Collection of samples

Fifteen samples of soil contaminated with engine oil remained heat-treated (80ºC for 10 min) to destroy all vegetative cells, then separately placed on nutrient agar dishes. Next, 24hr of incubation at 30ºC, colonies were collected and filtered by streaking on fresh nutrient agar.

Screening assays for bio-surfactant production

Mineral salt medium (MSM) were inoculated with 10% of each suspension isolates and grew aerobically for 7 days in shaker incubator, at 30ºC and stirred 150 rpm, composition (gm/l): 2.2 (Na_2HPO_4), 1.4 (KH_2PO_4), 0.6 ($MgSO_4.7H_2O$), 0.01 ($FeSO_4.7H_2O$), 0.05 (NaCl), 0.02 ($CaCl_2$), 0.02 (yeast extract), and (0.1) ml of trace element solution containing (gm/l): 2.32 ($ZnSO_4.7H_2O$), 1.78 ($MnSO_4.4H_2O$), 0.56 (H_3BO_3), 1.0 ($CuSO_4.5H_2O$), 0.39 ($Na_2MoO_4.2H_2O$), 0.42 ($CoCl_2.6H_2O$), 1.0 (EDTA), 0.004 ($NiCl_2.6H_2O$), and 0.66 (KI). pH of the medium was adjusted to 7.0 ± 0.2.

Different sources of carbon and nitrogen were supplied separately, like, (2% Vegetable oil), (1% Vegetable oil + 1% Glucose), (2% Glucose) (w/v) and $NaNO_3$ (1gm/l) (Ignacio *et al.,* 2015). Then surface tension was determined by (Surface tension / Sigma 703D / Finland) for cell-free supernatant following centrifuging the culture at 6000 rpm for 30 min.

Oil displacement examination

Petri dish (150 mm diameter) was supplied by added 15 µl of crude oil to 40 ml of DW. Next, 10 µl of cell-free supernatant was precisely located against the center of the oil layer, then after 30 sec, the diameter of the cleared halo region was measurement (Sriram *et al.,* 2011).

Biosynthesis of silver Nano-particles

Silver nanoparticle biosynthesis was done by Kumar *et al*. (2014), in brief, 50 ml of aqueous silver nitrate (100 mM) and 50 ml of culture supernatant were kept for 48 hr at room temperature. The bio-reduction of silver ions was observed at a period time by sampling (2 ml) of the interactive material. UV-visible spectra of those samples obtained and registering as function time of each reaction from 200 to 700 nm by (UV/VIS Spectrophotometer/Optima/Japan).

Biodegradation of petroleum wastes

Equilibrium volume of silver nanoparticle supplemented with bio-surfactant and engine oil waste was done and leave at room temperature for 48 hr, then estimated biodegradation of waste by (Gas-Chromatography (GC) / Master / Italy), after extraction with hexane.

Results and Discussion

Isolation of bacteria

Samples of soil contaminated with engine oil waste were heat-treated to destroy all vegetative cells and permit germination and growth of heat-resistant spores. Eighteen spore-former isolates from 15 samples of soil belong to genus *Bacillus,* were isolated based on microscopy (gram staining and spore staining) and morphological characteristics.

Oil spreading technique

The result of oil spreading technique for cell-free supernatant of *Bacillus* strain showed that MSM supplemented with (1% Vegetable oil + 1% Glucose) and MSM with (2% Vegetable oil) were the best in bio-surfactant production than MSM including (2% Glucose) as carbon source after incubated for 7 days in shaker incubator, at 30ºC and stirred with 150 rpm (Fig. 1). According to Sriram *et al*. (2011), the volume of bio-surfactant which required to see a clear detectable region on the oil layer termed minimum active dose (MAD).

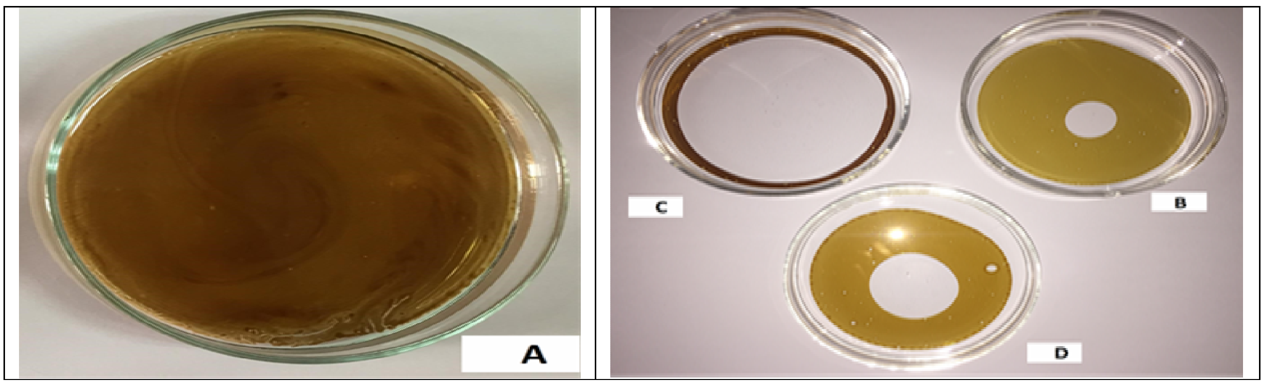

Fig. 1 : A distinct detectable zone on the oil layer indicates to bio-surfactant producing from MSM media supplemented with, (A) control, (B) glucose, (C) vegetable oil and glucose, (D) vegetable oil.

Bio-surfactant production

Table (1), illustrated that cell-free supernatant of *Bacillus* strain grown in the MSM media supplemented with (1% Vegetable oil + 1% Glucose) incubated at 30ºC for 7 days, was a good producer of bio-surfactant which is lowering the surface tension of water from 72 to 32.25 mN/m compared to others. Surface tension measurement was the method that reveals the production of bio-surfactant. But, it provided an indirect relation to the concentration of bio-surfactant in the liquid. The decrease of surface tension was indicated of the production of bio-surfactant by the microorganism. The greater reduction of surface tension would lead to the high concentration of bio-surfactant accessible (Rajesh, 2017).

Table 1 : Surface tension of bio-surfactant produced by *Bacillus* strain.

Different carbon source	Surface tension (mN/m)
Vegetable oil	32.92
Vegetable oil and glucose	32.25
Glucose	56.42

Bio-surfactant UV-visible spectroscopy was observed throughout the highest peak at 450 nm. The equal kind of results was achieved by Hoda *et al*. (2018). A similar result was also reported by Hina *et al*. (2018), they explained that silver nanoparticles were manufactured by cell-free supernatant of *Pseudomonas aeruginosa* after incubation for 72 hr.

The bio-surfactant would have played as the stabilization factor, which prevented the formation of aggregates and promoting the production moreover stability of nanoparticles under laboratory conditions. Structure of bio-surfactant plays a vital role in delimiting the morphology of nanoparticles. Those micelles are spherical and supported the formation of spherical nanoparticles. While bio-surfactants are natural surfactants recovered from microbial sources included of sugars and fatty acid moieties, they have higher biodegradability, lower toxicity, and great biological activities. Because of the bio-surfactants reduce the formation of aggregates due to the electrostatic force of attraction, they promote the stability of morphology and nanoparticles (Xie *et al.,* 2006).

Removal effect of engine oil waste by silver nanoparticles

The result showed that removing of petroleum waste by using (Bio-surfactant) and (Silver nanoparticle supplemented with bio-surfactant), after incubating for 48 hr at room temperature, and using GC Chromatography were (13.60% and 96.69%), respectively, according to the method (Zhu *et al.,* 2010) (Fig. 2, 3, 4 and 5).

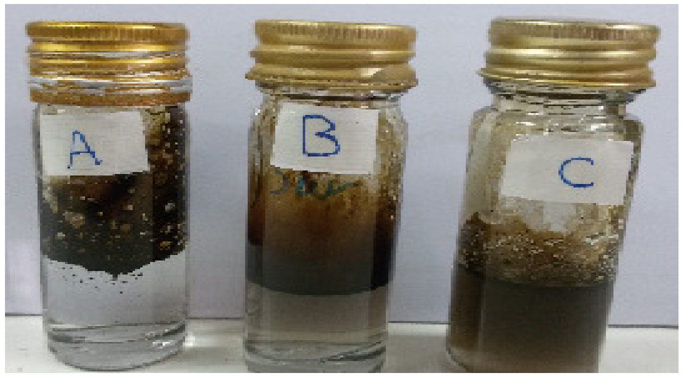

Fig. 2 : Removing of engine oil waste by (A) water and engine oil waste (Negative control) (B) bio-surfactant and engine oil waste (Positive control) (C) silver nanoparticle supplemented with bio-surfactant and engine oil waste, after 48 hr at room temperate.

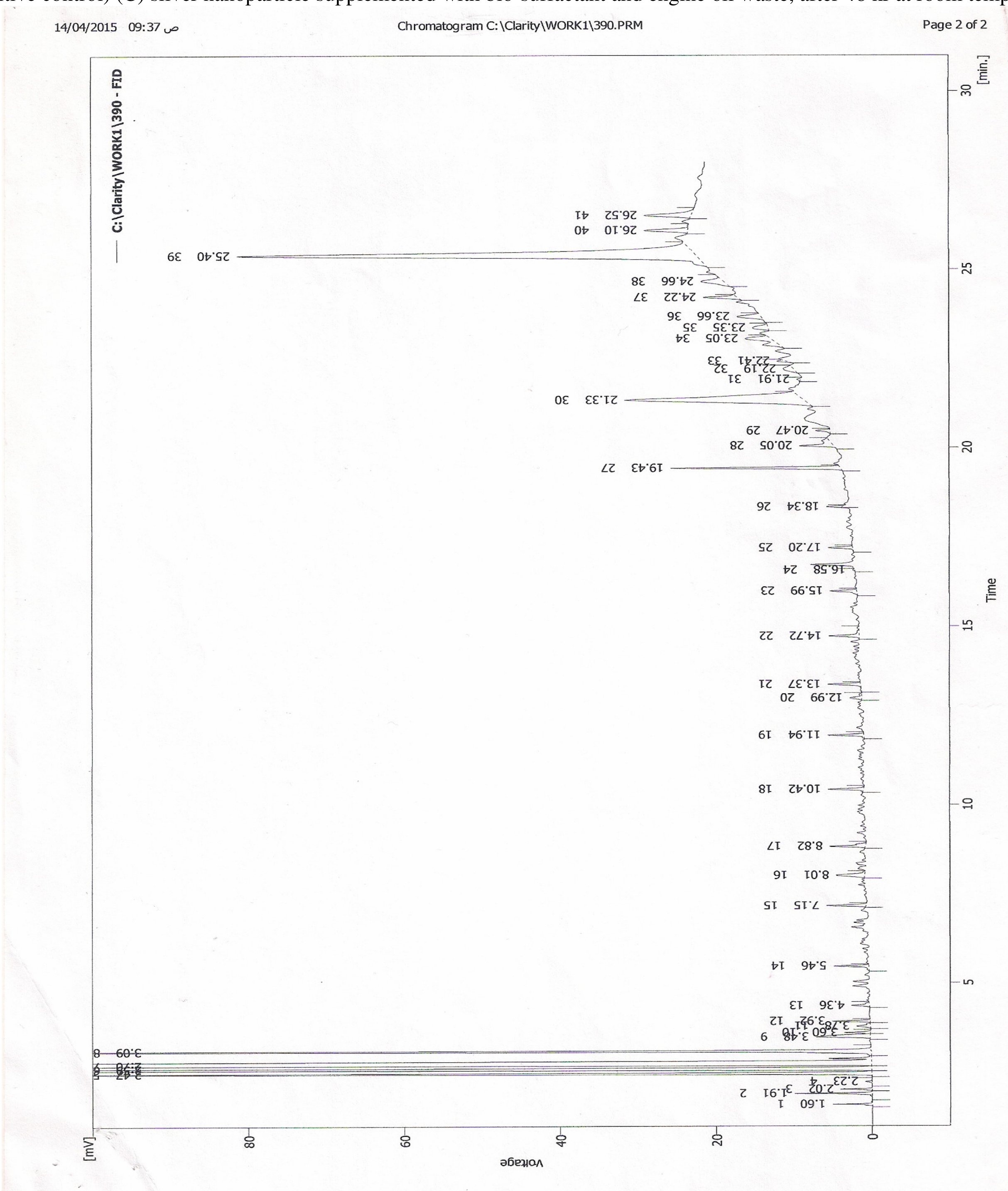

Fig. 3 : GC analytic for engine oil waste extracted with hexane before treatment.

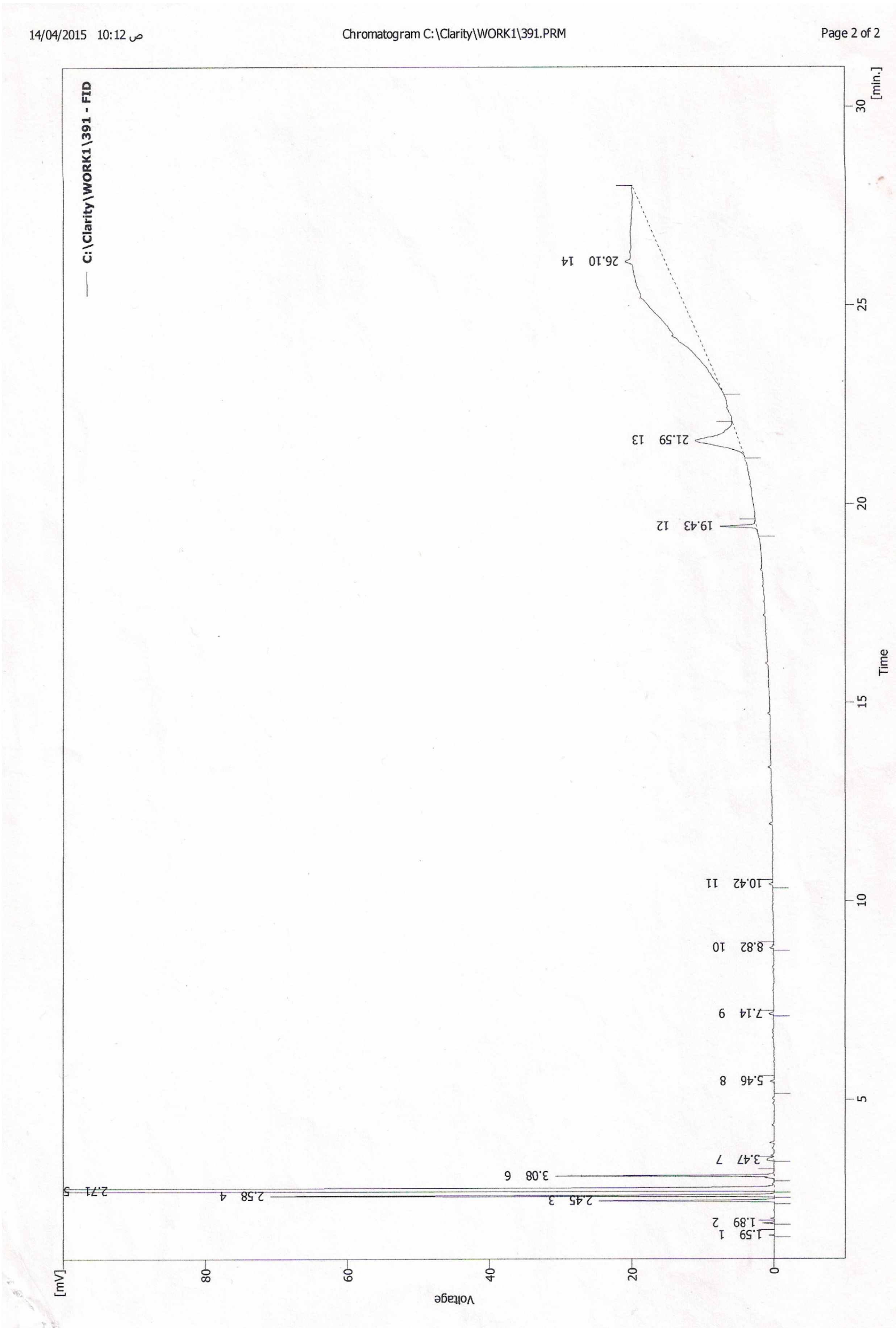

Fig. 4 : GC analytic for engine oil waste treating with bio-surfactant, after extracted with hexane.

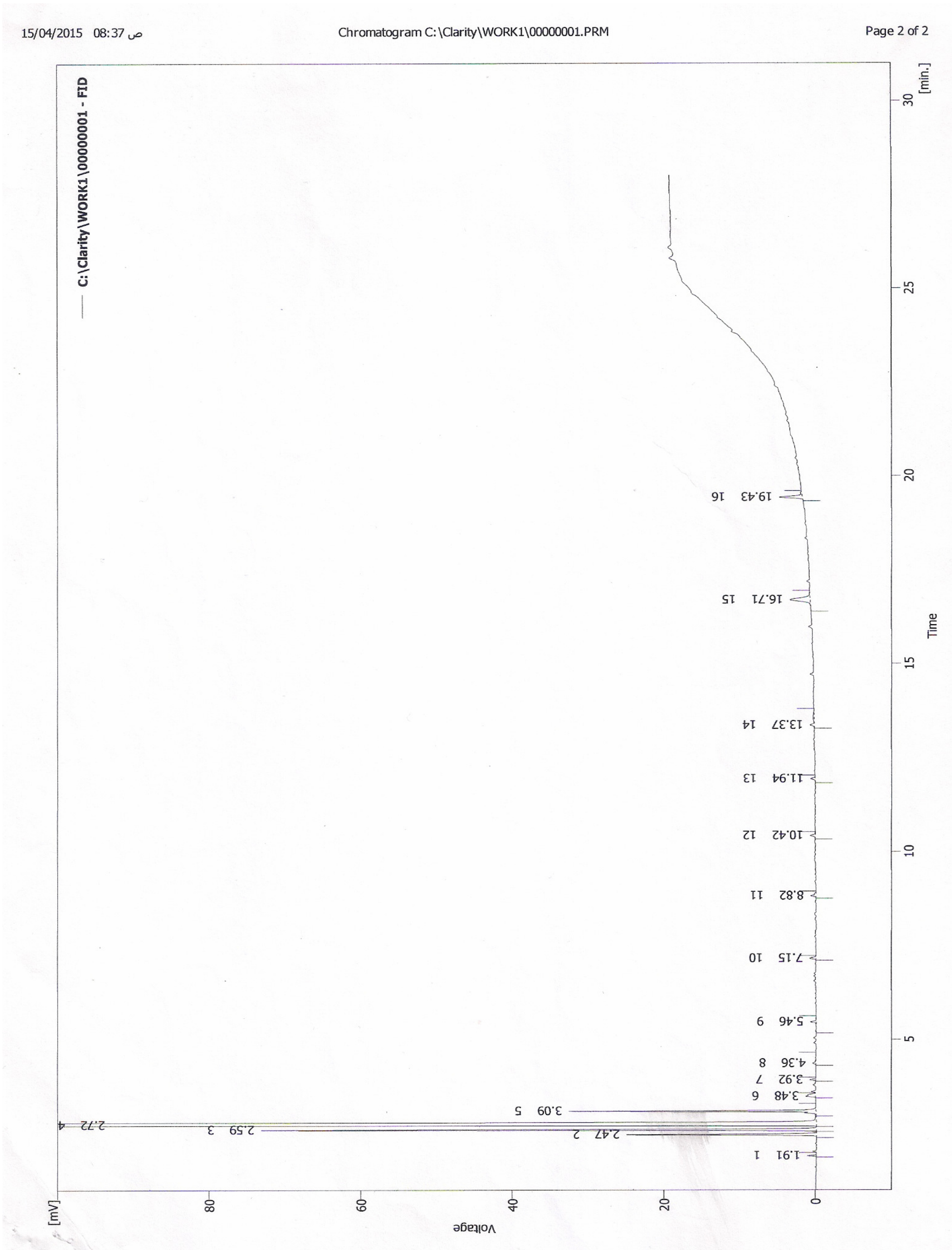

Fig. 5 : GC analytic for engine oil waste treating with silver nanoparticle supplemented with bio-surfactant, after extracted with hexane.

Conclusion

The advantage of low-cost, renewable and biodegradable bio-surfactants in replacement to toxic artificial surfactants, and it means ensuring an alternative and stabilization factor to prevent the formation of aggregates during the synthesis of inorganic nanoparticles under the laboratory conditions.

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