SUPPLEMEMTARY

Biosynthesis of *Pseudomonas aeruginosa* Mediated Silver Nanoparticles for the remediation of Crude Oil Contaminated Water

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Table S1. Morphological and biochemical characteristics of bacterial isolates in water samples collected from communities of Jones Creek oil field

Gram's Reaction	Shape	Citrate	Catalase	Methyl Red	Voges Proskauer	Spore Formation	Indole	MSA	Starch Hydrolysis	Oxidase	Glucose	Sucrose	Fructose	Sorbitol	Arabinose	Urease	Presumptive Bacteria identified
-	R	+	+	-	-	-	+	-	-	+	-	+	-	-	-	-	*Pseudomonas aeruginosa*
-	R	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	*Pseudochrobactrum asaccharolyticum*
+	R	+	+	+	-	+	-	-	+	-	+	-	-	+	-	-	*Bacillus megaterium*
+	C	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	*Micrococcus luteus*
+	R	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	*Bacillus subtilis*
-	R	-	+	+	-	-	-	-	+	-	-	+	-	-	-	-	*Escherichia coli*
+	R	+	+	-	-	+	-	-	+	-	-	+	-	+	+	-	*Bacillus cereus*
+	C	-	+	-	+	-	-	-	-	+	+	-	-	-	-	+	*Macrococcus caseolyticus*
+	R	+	+	+	-	+	-	-	+	-	-	+	-	-	-	-	*Bacillus thuringiensis*

R= Rod, C= Cocci, - = Negative, + = Positive, MSA= Mannitol Salt Agar

Table S2. Counts of bacterial isolates from water during 28 days screening in crude oil

Bacterial Isolates	Bacterial counts (x 10^4 cfu/mL) Time (Days) 7	14	21	28
Pseudomonas aeruginosa	6.00±2.00^{c}	TNTC	18.00±3.61^{d}	6.33±2.52^{c}
Pseudochrobactrum asaccharolyticum	5.00±2.00^{bc}	TNTC	23.00±4.36^{e}	6.00±3.61^{bc}
Bacillus megaterium	3.00±0.00^{ab}	6.33±1.53^{bc}	11.67±1.53^{c}	1.67±0.58^{a}
Micrococcus luteus	2.33±1.53^{ab}	4.33±0.58^{b}	3.33±1.53^{a}	3.00±1.00^{ab}
Bacillus subtilis	4.00±1.00^{abc}	11.00±1.73^{d}	10.00±1.73^{bc}	2.33±0.58^{a}
Escherichia coli	1.33±0.58^{a}	2.00±1.00^{a}	2.33±1.15^{a}	1.00±0.00^{a}
Bacillus cereus	3.33±1.53^{abc}	7.00±2.65^{c}	6.00±2.00^{ab}	2.33±1.53^{a}
Macrococcus caseolyticus	5.00±1.00^{bc}	TNTC	12.00±2.00^{c}	3.67±1.53^{abc}
Bacillus thuringiensis	3.33±2.08^{abc}	4.67±1.53^{bc}	2.33±0.58^{a}	1.67±1.15^{a}

Values are expressed in mean ± standard error of mean of triplicate determination. Values with different alphabet on the same row are statistically significant different at $p<0.05$.
Keys: TNTC= Too Numerous to Count, cfu/mL = Colony FormingUnits per mL.

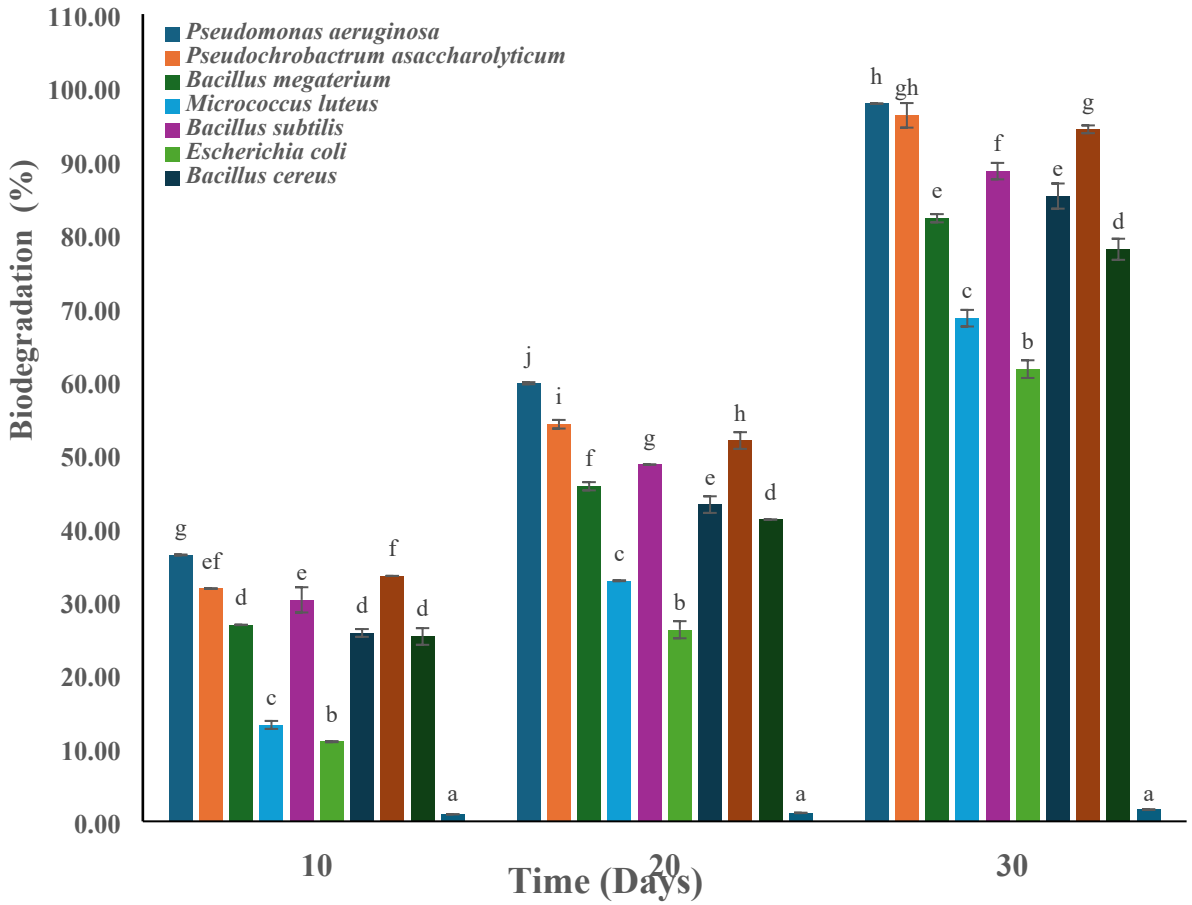

Fig. S1. Crude oil biodegradation by bacterial isolates

Table S3. NCBI blast result revealing the identity of each bacteria sequence

Sample ID	Scientific Name	Max Score	Total Score	Query Cover	E-value	Percentage Identity	Acc. Len	Accession
A	*Pseudomonas aeruginosa*	2529	10094	100%	0	99.43%	1394	ON600888
B	*Pseudomonas aeruginosa*	2556	10205	100%	0	99.78%	1394	ON600889
C	*Pseudochrobactrum asaccharolyticum*	2468	2468	100%	0	99.85%	1342	ON600890
D	*Macrococcus caseolyticus*	2580	2580	99%	0	99.72%	1401	ON600891

NCBI = National Centre for Biotechnology information, BLAST = Basic Local Alignment Search Tool.

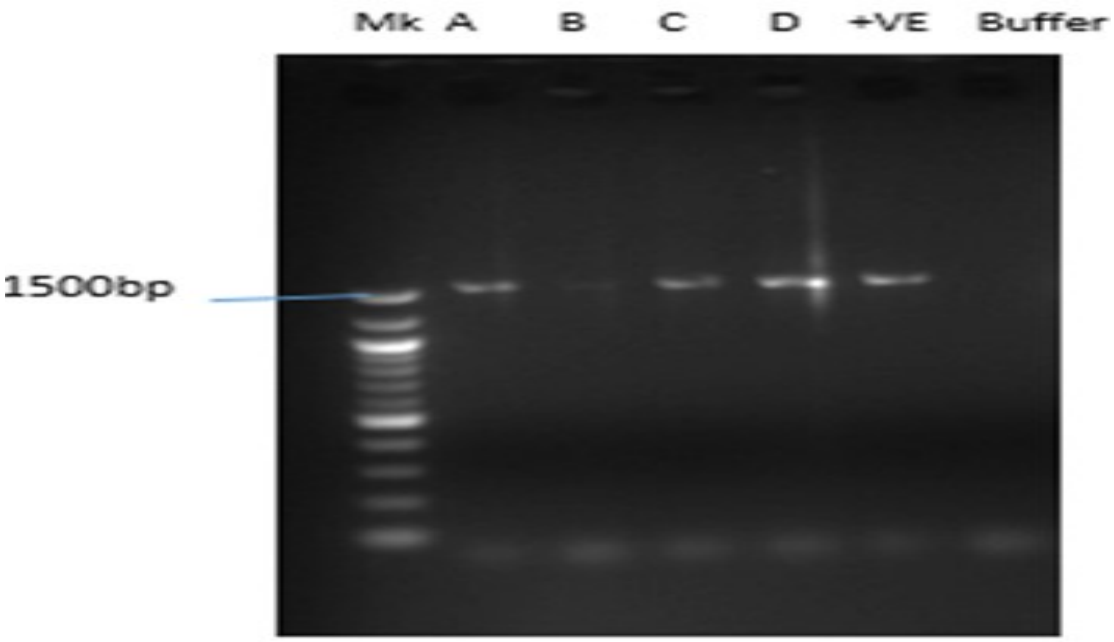

Plate S1. Agarose gel electrophoretic image of Sslected bacterial isolates

Plate S2. Colour variations in the reaction mixtures of the bacterial supernatant
Keys: A= Before Incubation, B= after incubation.

Plate S3. Controls using culture supernatant of bacterial strain alone and $AgNO_3$ solution alone Keys: E1= culture Supernatant of *Pseudomonas aeruginosa* strain AgA, $AgNO_3$ = silver nitrate solution alone.

Table S4. Model fit for adequacy

Std. Dev.	0.8705	R^2	0.9190
Mean	95.73	Adjusted R^2	0.8683
C.V. %	0.9093	Predicted R^2	0.7785
		Adeq Precision	14.8176

Table S5. Analysis of variance for the response

Source	**SS**	***df***	**PAE *MS***	**F-Value**	**P-Value**
Model	137.49	10	13.75	18.14	< 0.0001
A-CT	21.12	1	21.12	27.87	< 0.0001
B-SP	4.11	1	4.11	5.42	0.0334
C-Dosage	1.74	1	1.74	2.30	0.1492
D-Temp	15.48	1	15.48	20.43	0.0003
AB	12.85	1	12.85	16.96	0.0008
AC	17.43	1	17.43	23.00	0.0002
AD	4.33	1	4.33	5.71	0.0295
BC	48.23	1	48.23	63.65	< 0.0001
BD	9.36	1	9.36	12.36	0.0029
CD	2.84	1	2.84	3.75	0.0708
Residual	12.13	16	0.7578		
Lack of Fit	10.52	14	0.7513	0.9353	0.6303
Pure Error	1.61	2	0.8033		
Cor Total	149.62	26			

CT: Contact time, SP: Stirring speed, SS: Sum of squares, df: Degrees of freedom, MS: mean of squares, P-Value: Probability, PAE: AgNPs synthesized using*Pseudomonas aeruginosa* strain AgA.

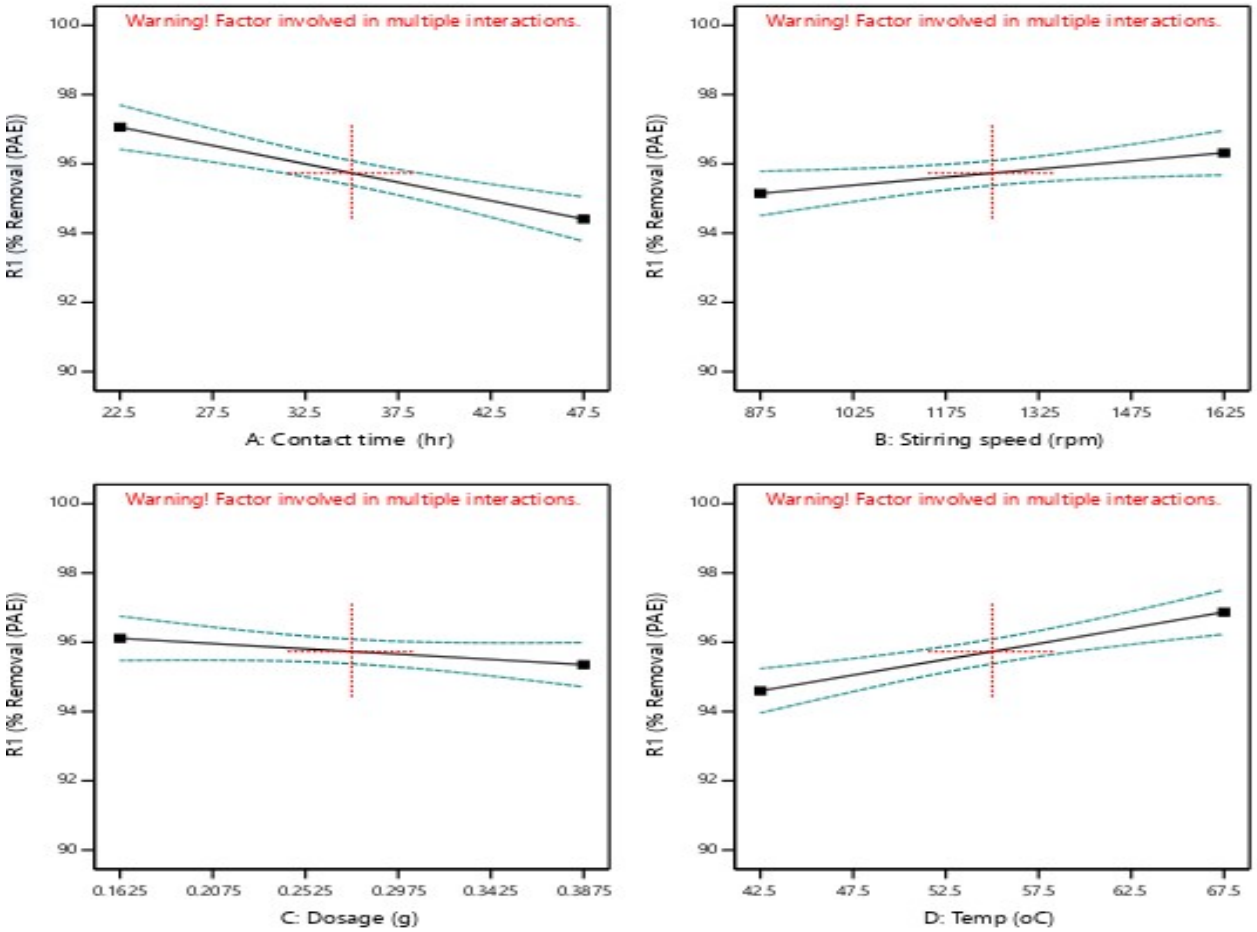

Fig. S2. Effect of the individual Factors on TPH Removal using *Pseudomonas aeruginosa* strain AgA

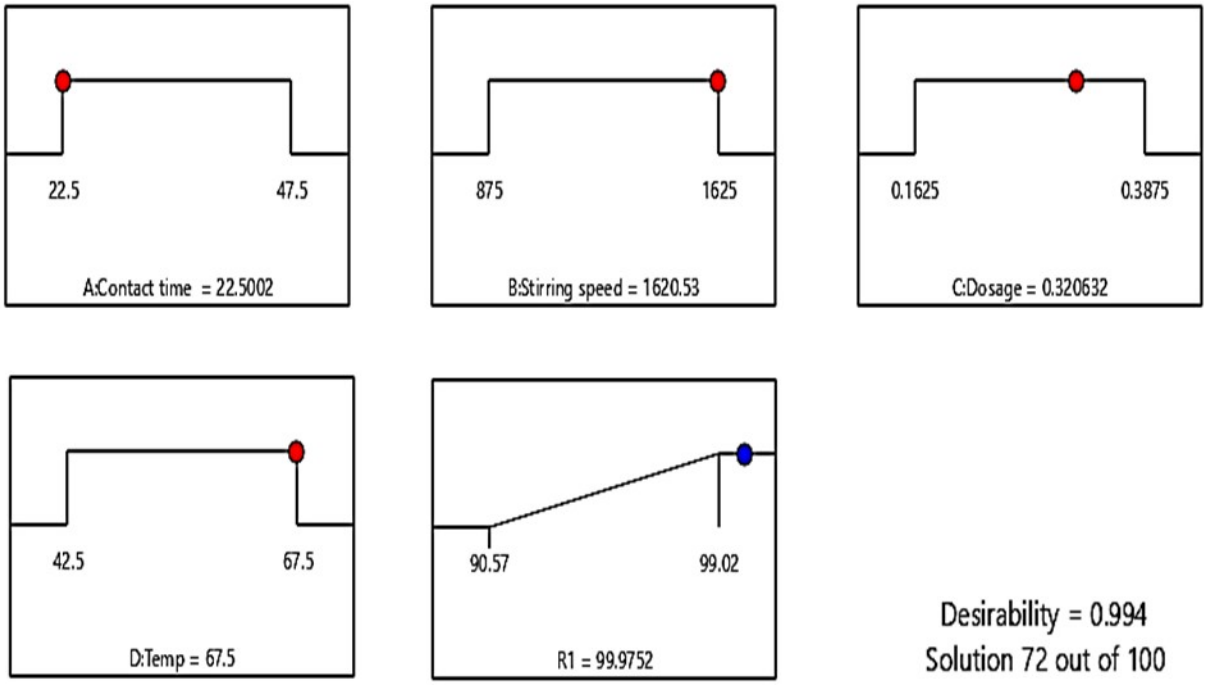

Fig. S3. Optimal factor and response predictive and desirability chart

Table S6. Validation of predicted values of the TPH removal using the biosynthesized AgNPs

Responses	Predicted value	Experimental value	Difference	% change
PAE	99.975	98.750	1.225	1.22

PAE: AgNPs synthesized using *Pseudomonasaeruginosa* strain AgA

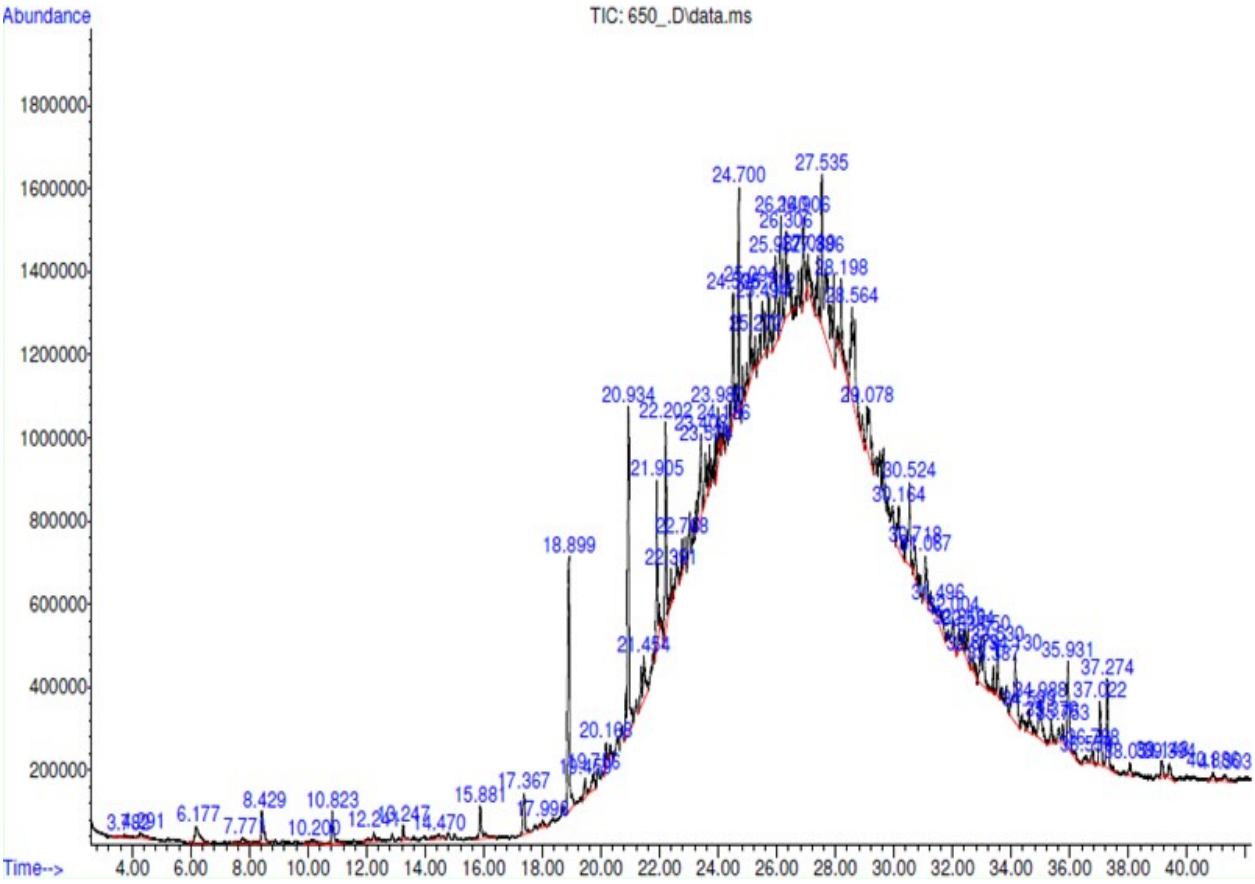

Fig. S4. Chromatogram of the hydrocarbons extracted from the untreated composite water sample

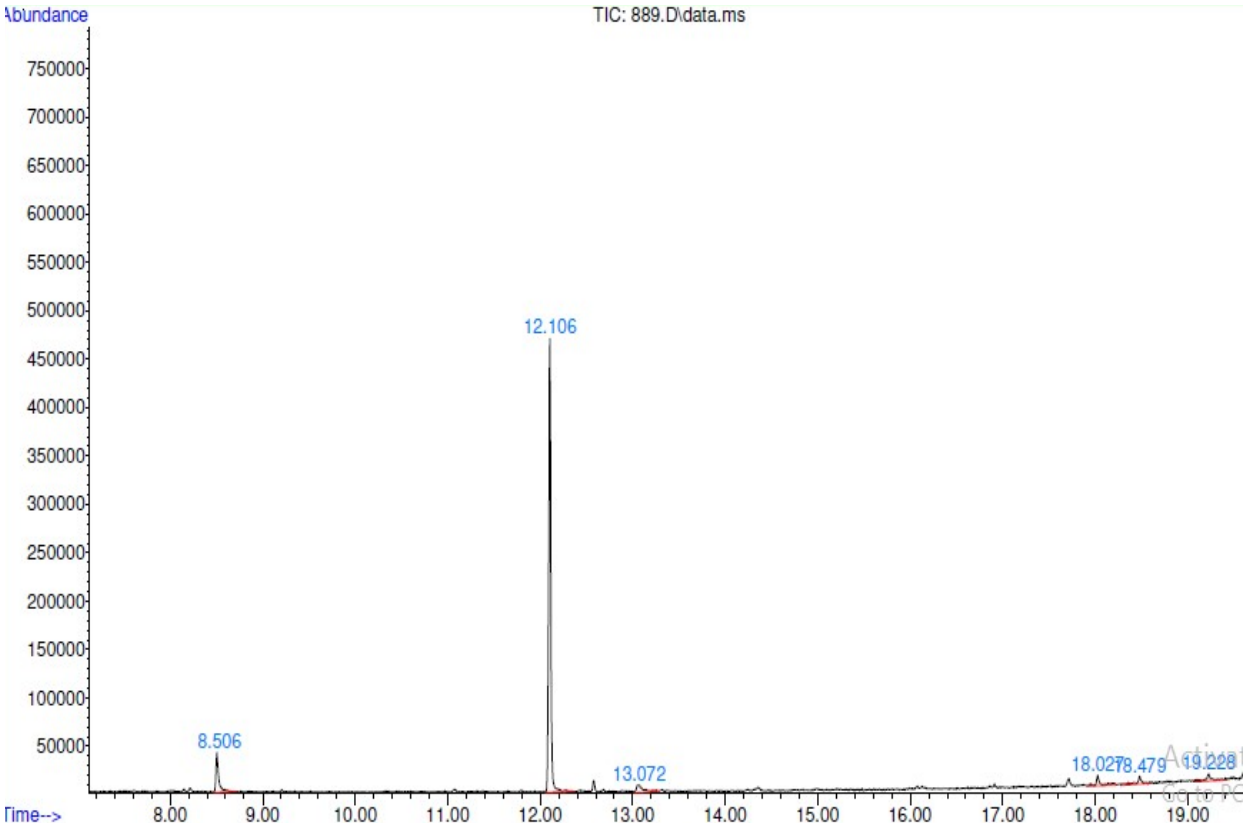

Fig. S5. Chromatogram of the biodegraded crude oil by the biosynthesized AgNP