Annexure -VIII

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002.

Annual/Final Report of the work done on the Major Research Project.

(Report to be submitted within 6 weeks after completion of each year)

1. Project report No. 1st /2nd /3rd/Final : Final year
2. UGC Reference No.F : MRP-MAJOR-MICR-2013-31825
3. Period of report : from 01/07/2015 to 30/06/2018
4. Title of research project : Microbiological influenced corrosion behaviour of aerobic/anaerobic microbial consortia with special reference to petroleum crude oil industry
5. (a) Name of the Principal Investigator : Dr. A. Rajasekar
   (b) Dept. : Biotechnology
   (c) University/College where work has progressed : Thiruvalluvar University
6. Effective date of starting of the project : 01/07/2015
7. Grant approved and expenditure incurred during the period of the report:
   a. Total amount approved Rs. 14,64,678/-
   b. Total expenditure Rs 14,64,678/-

c. Report of the work done: (Please attach a separate sheet)

i. Brief objective of the project:

a) Investigating Microbially- induced corrosion (MIC) in crude oil wells/ transporting pipelines

b) Bacterial enumeration and 16SrDNA geneidentification of crude oil product degrader including aerobic/ anaerobic bacteria.

c) Biodegradation of hydrocarbon and its influence on corrosion

d) Development of code of practice for evalution of suitable eco-friendly and effective corrosion inhibitors/ biocides formulation for petroleum industry.

ii) Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication :

a) Investigating Microbially- induced corrosion (MIC) in crude oil wells/ transporting pipelines

Samples (Production water and crude oils from oil well head and storage tank) were collected from **Oil and Natural Gas Corporation (ONGC),** Cauvery Asset, **karaikal-** Pondicherry**.** Samples are collected from two different locations called **Adiyakamangalam and Kamalapuram** based on the seriousness of the oil field problems due to corrosion**.** The crude oil was flow out from the well by using natural pressure through X mass tree. The water present in the oil well samples was observed as in the range of 30% - 50% v/v.

b) Bacterial enumeration and 16SrDNA geneidentification of crude oil product degrader including aerobic/ anaerobic bacteria.

The aerobic/ anaerobic bacterial was enumerated by using pour plate technique with appropriate medium. Nutrient agar, minimal salt medium, Sulphate API Agar, Iron oxidizing medium, manganese oxidizing agar and Thiobacillus agar were used to enumerate the heterotrophic bacteria, iron bacteria, acid producers, and manganese-oxidizing bacteria, respectively. After the pour plate with the appropriate medium and then the plates were incubated at different temperature, 37^0C, 40^0C and 55^0C.

c) Biodegradation of hydrocarbon and its influence on corrosion

Biodegradation studies were carried out with selected bacterial strains as mixed consortium. Degradation of crude oil was evaluated following the protocol described by Rahman et al. (2002). Pre-cultured mixed consortia (initial load about 2.1 x 10^4 CFU mL^{-1}) were transferred in a 250 mL Erlenmeyer screw cap flask, each containing 100 mL of BH broth supplemented with 1% (v/v) sterile crude oil as carbon source. The flasks were incubated at 37 °C for 20 days at 200 rpm. All experiments were performed in triplicate. Biodegradation of crude oil hydrocarbons was examined by GC-MS and shown in Figure 1. Cell viability was checked using the epi-fluresance microscopy (Fig. 2).

D) Development of code of practice for evaluation of suitable eco-friendly and effective corrosion inhibitors / biocides formulation for petroleum industry

Microbiologically Influenced Corrosion (MIC) behavior of carbon steel by the bacterial consortia was investigated. With previous corrosion studies four bacterial strains were identified as potential corrosion enhancer in the oil filed environments. To inhibit their growth different types of green and synthesized inhibitor were tested against those corrosive bacterial strains. Biofilm formed over metal surface was observed using scanning electron microscope (SEM) (Fig. 3) and Surface pit was also visualized using SEM (Fig. 4). Electrochemical impedance spectroscopy including AC-impedance (Fig. 5) and polarization

(Fig. 6) were carried out to confirm the nature of the corrosion takes place in the metal surface (Table 1). Corrosion inhibition efficiency was found about 70-80% in presence of neem as inhibitor (Table 2). X-ray powder diffraction was carried out to known the nature of the oxide present in the rust particles

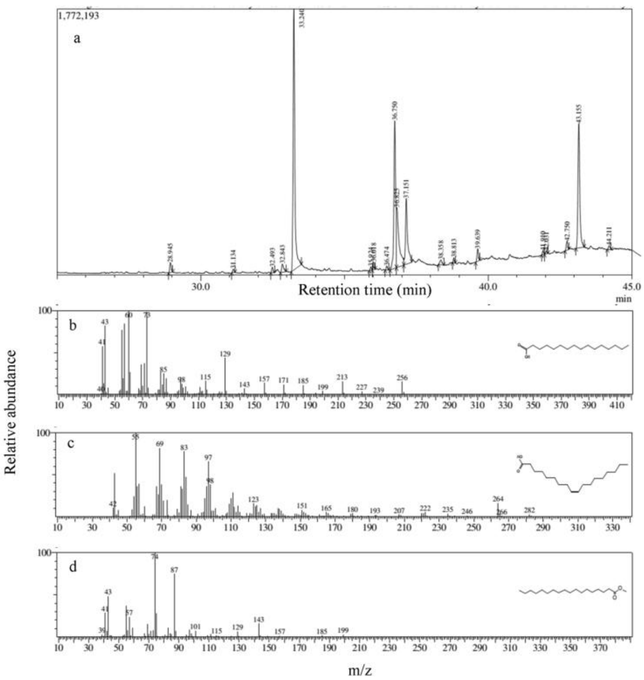

Fig. 1. GC-MS analysis of biosurfactant from *S. parvus* B7 (**a**) GC spectrum of biosurfactant; (**b**) Mass spectra of n-hexadecanoic; (**c**) Mass spectra of octadecanoic acid and (**d**) Mass spectra of octadecanoic acid, methyl ester.

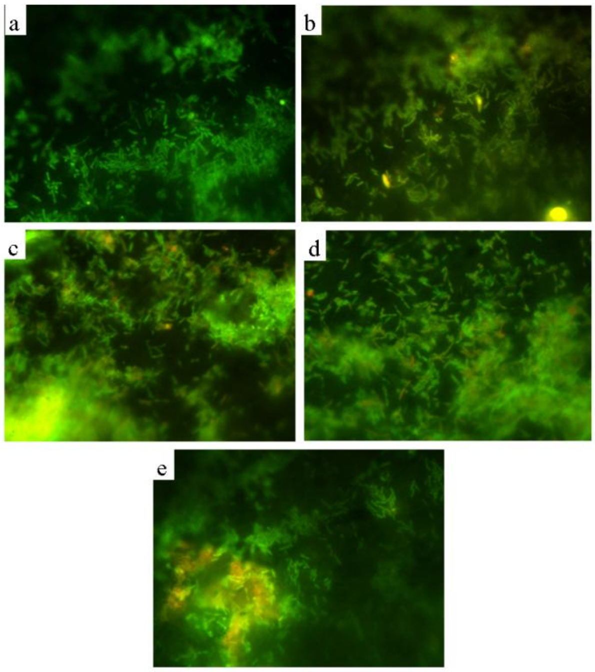

Fig.2. Epi-fluorescence micrograph of bacterial biofilm (a) 2nd day (b) 4th day (c) 6th day (d) 8th day and (e) 10th day.

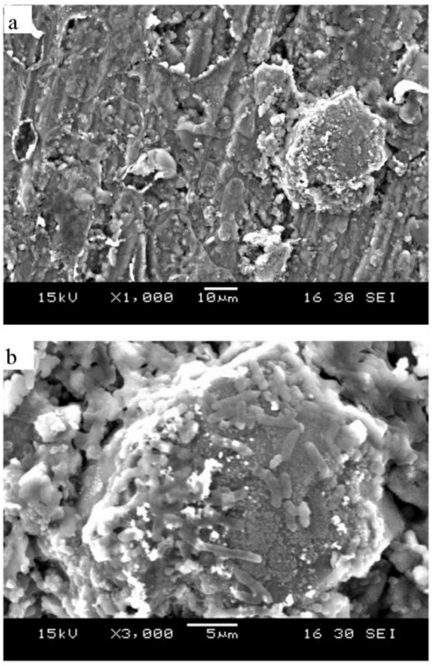

Fig. 3. SEM micrograph of biofilm formation on carbon steel API 5LX surface coupon exposed in bio-corrosion studies; (a) Over view of the biofilm on metal surface and (b) Magnified view of the biofilm and bacterial attachments.

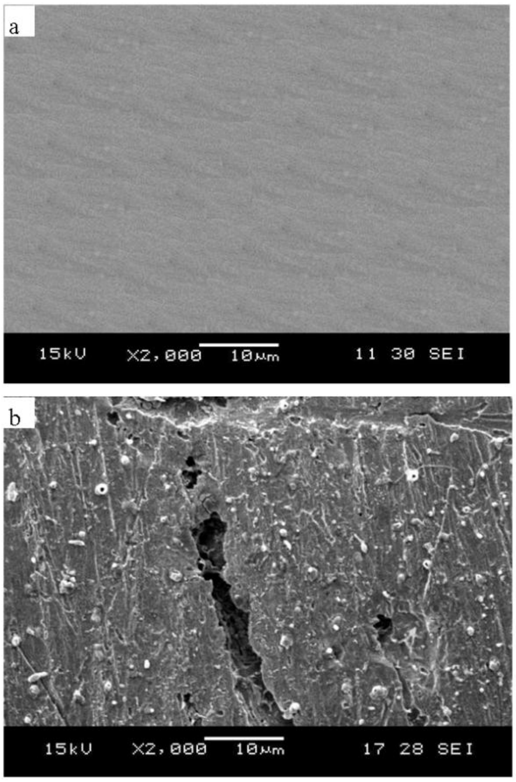

Fig. 4. SEM micrograph of typical pits formed on surface of the carbon steel API 5LX immersed in bio-corrosion studies; (a) abiotic control (bare metal) and (b) Mixed consortia.

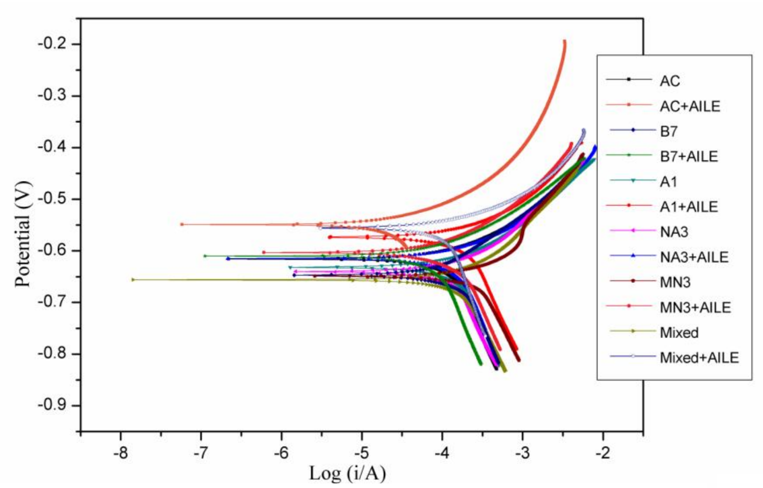

Fig. 5. The potentiodynamic polarisation curves of the carbon steel API 5LX in presence and absence of bacterial strains and neem extract. Note: A1 - *B. subtilis*, B7 - *S. parvus*, NA3 – *P. stutzeri*, MN3- *A. baumannii*, AC- Abiotic control, AILE- *Azadirachta indica* leave extract.

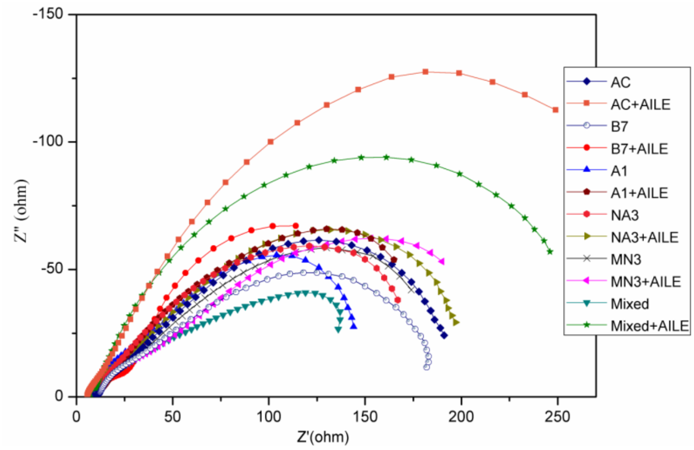

Fig. 6. Electrochemical impedance illustration of the carbon steel API 5LX in the presence and absence of bacterial strain and neem inhibitor. Note: A1 - *B. subtilis*, B7 - *S. parvus*, NA3 – *P. stutzeri*, MN3- *A. baumannii*, AC- Abiotic control, AILE- *Azadirachta indica* leave extract.

Table 1 Corrosion rate and inhibition efficiency for the carbon steel API 5LX in the presence and absence bacterial strains and neem inhibitor.

Systems	Carbon steel API 5LX		
	Weight loss (mg)	Corrosion rate (mm/y)	Corrosion Inhibition efficiency (%)
I - Abiotic Control	16.0 ± 1	0.060	-
II- Abiotic control with AILE	3.1± 0.5	0.012	81
III – *S. parvus* B7	40.2 ± 2	0.150	-
IV – *S. parvus* B7 with AILE	3.5 ± 0.5	0.013	78
V – *B. subtilis* A1	38.6± 2	0.114	-
VI – *B. subtilis* A1 with AILE	4.4 ± 0.5	0.017	72
VII- *P. stutzeri* NA3	36.4± 1	0.136	-
VIII- *P. stutzeri* NA3 with AILE	4.0± 0.5	0.015	75
IX – *A. baumannii* MN3	32.8± 1	0.122	-
X - *A. baumannii* MN3 with AILE	4.2± 0.5	0.016	74
XI - Mixed consortia	48.2 ± 2	0.179	-
XII - Mixed consortia with AILE	4.5 ± 0.5	0.017	72

Note: AILE- *Azadirachta indica* leave extract.

Table 2 Polarization and impedance parameters for carbon steel API 5LX in the presence/absence bacterial strains and neem extract

Systems	polarization data				impedance data	
	I_{corr} (A/cm^2)	E_{corr} (V)	β_a (mV/dec)	β_c (mV/dec)	R_{ct} ($\Omega\cdot cm^2$)	R_s (ohm)
I - Abiotic Control	1.9 x 10^{-4}	-596	7.53	2.29	16.13	9.65
II- Abiotic control with AILE	1.0 x 10^{-4}	-570	7.22	1.82	20.2	9.98
III – *S. parvus* B7	2.0 x 10^{-4}	-647	9.45	2.84	11.15	8.69
IV – *S. parvus* B7 with AILE	1.3 x 10^{-4}	-610	7.56	2.20	15.95	9.21
V – *B. subtilis* A1	2.3 x 10^{-4}	-632	8.49	2.87	12.01	7.53
VI – *B. subtilis* A1 with AILE	2.0 x 10^{-4}	-594	7.95	2.38	17.83	9.45
VII- *P. stutzeri* NA3	2.6 x 10^{-4}	-640	8.93	2.97	10.99	7.95
VIII- *P. stutzeri* NA3 with AILE	2.2 x 10^{-4}	-615	8.09	2.18	16.42	9.36
IX – *A. baumannii* MN3	1.7 x 10^{-4}	-649	9.55	3.09	11.54	7.49
X - *A. baumannii* MN3 with AILE	1.2 x 10^{-4}	-603	7.87	2.48	17.56	9.22
XI - Mixed consortia	3.3 x 10^{-4}	-656	10.18	3.53	11.1	7.01
XII - Mixed consortia with AILE	1.6 x 10^{-4}	-586	7.36	2.20	19.53	9.74

Note: AILE- *Azadirachta indica* leave extract.

Publications

1. P. Parthipan, P. Elumalai, J. Narenkumar, LL Machuca, K. Murugan, OP Karthikeyan, **A.Rajasekar**. *Allium sativum* (garlic extract) as a green corrosion inhibitor with biocidal properties for the control of MIC in carbon steel and stainless steel in oilfield environments. International biodeterioration & biodegradation 132 (2018) 66-73. **(IF: 3.5)**
2. P. Parthipan, J. Narenkumar, P. Elumalai, P.S. Preethi, A.U.R. Nanthini, A. Agrawal **A. Rajasekar**, Neem extract as a green inhibitor for microbiologically influenced corrosion of carbon steel API 5LX in a hypersaline environments, J.Mol.Liq. 240 (2017) 121–127. doi.org/10.1016/j.molliq.2017.05.059. **(IF: 4.5)**
3. P.Elumalai,P.Parthipan, J.Narenkumar, RK. Sarankumar, OP. Karthikeyan, **A.Rajasekar**, Influence of Thermophilic Bacteria on Corrosion of Carbon Steel in Hyper Chloride Environment. Int J Environ Res. 11(3) (2017) 339–347. **(IF: 1.0)**
4. J.Narenkumar**,** N.Ramesh, **A.Rajasekar**. Control of corrosive bacterial community by biocide bronopol in industrial water system. 3 Biotech. 8(1) (2018.) 55. **(IF: 1.4)**
5. J.Narenkumar, K. Sathishkumar, RK. Sarankumar, K. Murugan, **A.Rajasekar** , An anticorrosive study on potential bioactive compound produced by *Pseudomonas aeruginosa* TBH2 against the biocorrosive bacterial biofilm on copper metal, J.Mol.Liq. 243 (2017) 706-713, doi:10.1016/j.molliq.2017.08.075. **(IF: 4.5)**
6. J.Narenkumar, K.Sathishkumar, A.Selvi, R.Gobinath, K.Murugan, **A.Rajasekar**. Role of calcium depositing bacteria *Agrobacterium tumefaciens* and its influence on corrosion of different engineering metals used in cooling water system. 3 Biotech 7 (2017) 374. **(IF: 1.4)**

7. J.Narenkumar**,** P.Parthipan, J.Madhavan, K. Murugan, A.Suresh, **A. Rajasekar**. Bio-engineered Silver Nanoparticles: Potent Anti-Corrosive Inhibitor against Corrosion of Mild Steel in Cooling Towers. Environmental Science and Pollution Research. 25(6) (2018) 5412-5420. (**IF: 2.8)**
8. J. Narenkumar, P. Parthipan, AUR. Nanthini, G. Benelli, K. Murugan and **A. Rajasekar**, Ginger extract as green biocide to control microbial corrosion of mild steel. 3 Biotech. (2017) 7(2): 133. doi: 10.1007/s13205-017-0783-9. (**IF: 1.4)**
9. K.Sathishkumar, J.Narenkumar, J.Madhavan, K.Murugan, **A.Rajasekar**., Electrochemical decolorization and biodegradation of tannery effluent for reduction of chemical oxygen demand and hexavalent chromium**.,** Journal of Water Process Engineering, 20, (2017) 22-28.
10. J. Narenkumar, J. Madhavan, M. Nicoletti, G. Benelli, K. Murugan, **A. Rajasekar**, Role of bacterial plasmid on biofilm formation and its influence on corrosion of engineering materials. J Bio TriboCorros. (2016) 2:24.
11. K.Sathishkumar, J.Narenkumar**,** A.Selvi, K.Murugan, R.Babu Janarthanam, **A.Rajasekar**., Treatment of soak liquor and bioelectricity generation in dual chamber microbial fuel cell., Environmental Science and Pollution Research 25 (2018) 11424. **(IF: 2.8)**

iii) Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons

iv) Please indicate the difficulties, if any, experienced in implementing the
Project: No

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet.

vi. If the project has been completed, please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to University Grants Commission.

The overall project summaries that *Azadirachita indica* (AILE) and *Allium sativum* (GAE) can act as an efficient, eco-friendly green corrosion inhibitor with biocidal properties for control of MIC on carbon steel API 5LX in hypersaline environment. From the growth curve, the total viable counts of the bacterial cells in corrosion systems with both green inhibitors were highly reduced to 10^0. It reveals that, both green inhibitors have an antibacterial effect against all the bacterial biofilm on carbon steel surfaces. Weight loss and electrochemical analysis showed that bacterial strains *S. parvus* B7, *B. subtilis* A1, *P. stutzeri* NA3 and *A. baumannii* MN3 increased the corrosion rate of carbon steel. This is the first report to show degradation of crude oil by *S. parvus* B7 and its influence (acceleration) on the corrosion of carbon steel API 5LX in oil reservoir. In oil reservoir degraded organic compounds in crude oil encourage the growth of bacteria and subsequently, bacteria accelerate the corrosion reaction by forming Fe_2O_3. These bacterial strains cause carbon steel deterioration severely in the corrosive produced water medium by forming biofilm over the metal surface. At the same time these bacterial strains were very sensitive to the both green inhibitors (AILE and GAE). AILE and GAE suppressed the corrosion mechanisms in carbon steel and the inhibition efficiency were ranged as 72-81%.

The presence of biocidel compounds azadirachtin and other phytochemical in the AILE and diallyl disulphide in GAE could be playing an important role in the inhibitory mechanism of biocorrosion. The active compounds neem and garlic co-ordinates with iron on metal surface and form a protective film. Based on these findings *Azadirachita indica* and *Allium sativum* can be suggested as a green inhibitor with biocidal properties for control of MIC in crude oil reservoir environment including transporting pipeline and storage tanks.

vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any

b) J. Narenkumar, Reg no: Ph.D/ 008/2015

SIGNATURE OF THE PRINCIPAL INVESTIGATOR **REGISTRAR/PRINCIPAL**

(Seal)