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Assessment of Microbiologically Influenced Corrosion of Metals in Biodiesel from *Jatropha curcas*

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ABSTRACT

Use of biodiesel provides promising opportunities for immediately addressing our energy security issues. Being plant based, biodiesels are more hygroscopic and more readily biodegraded than fossil fuels and the introduction and widespread use of biodiesels in the existing fuel infrastructure may lead to increased problems of microbiologically influenced corrosion (MIC). Effective control and prevention of MIC require an underlying knowledge of the microbes that survive in biodiesel and the ingredients that help in their growth. This paper discusses the corrosion of aluminum and copper in *Jatropha curcas* biodiesel and its blends with commercial diesel under the influence of bacteria isolated from the sediments of stored jatropha biodiesel by mass loss measurements. The results indicate that both the metals show highest corrosion rate in B100.

Keywords: hygroscopic, MIC, *Jatropha curcas* biodiesel, aluminium, copper, biodiesel blends

INTRODUCTION

Depleting fossil fuel supplies, soaring oil prices and growing concern about green house gas emissions are some of the issues that have prompted many countries to look for renewable energy sources. While looking for new energy sources, biofuels can make a significant contribution to satisfying the needs of society for energy. Biodiesel holds a great promise as an environmentally friendly fuel alternative for the fast depleting fossil fuels.[1] Biodiesels are methyl or ethyl esters derived from a wide variety of renewable resources such as vegetables oils, animal fats and wastes. The choice of feed for biodiesel is country specific and depends on availability. In India, non-edible oil is the preferred choice for the production of biodiesel since the demand for edible oil exceeds the domestic supply. Being a plant of tropics, *Jatropha* *curcas* is an ideal plant for biodiesel production, since it is a drought and pest-tolerant plant which can grow in arid and semi-arid region[2] and its oil content is higher than that of other oil crops.[3]

Though biodiesel has many societal benefits, the compatibility of biodiesel with metallic materials is of great concern.[4,5] By virtue of their biodegradability, use of biodiesel will lead to problems of microbial spoilage and corrosion.[6,7] Microbiologically influenced corrosion (MIC) is an electrochemical process in which microorganisms are able to initiate, facilitate or accelerate the corrosion reaction[8] and poses serious problems in oil, gas and shipping industries.Corrosion problems encountered in petroleum product transporting pipeline was studied by Maruthamuthu *et al*.[9] The effect of bacterial contamination on the corosion of aluminum alloy used in the aeronautical industry was investigated by Rajasekar *et al*.[10]

Being hygroscopic in nature, biodiesels offer the perfect environment for the microorganisms to thrive by increasing the water content within the fuel and nutritive substances available leading to MIC of metals which affects fuel distribution, storage supply and end use.MIC in biodiesel was evaluated by Lee *et al*.[11] and found that B100(100% biodiesel) had the highest propensity for biofouling.Investigation of microbial stability of biodiesel blends showed that a variety of microorganisms were involved in fuel degradation.[12] Only limited works are available on the nature of microbes that thrive in bio-diesel and the occurrence of MIC in the presence of biodiesel and biodiesel petrodiesel blends. Hence this work aims to isolate bacteria from the sediments of stored jatropha biodiesel and study its influence on the corrosivity of jatropha biodiesel and its blends with commercial diesel on aluminium and copper, which are widely used for making automotive engine parts like fuel pump, fuel injector, filters and piston.

EXPERIMENTAL PROCEDURE

Bacterial Sample Collection, Enumeration and Isolation of Bacteria

In continuation of the previous work on the corrosivity of jatropha oil[13] and jatropha biodiesel, [14] the sediment at the bottom of the container having a two year old sample of *Jatropha curcas* biodiesel (JBD) was collected in a sterile container. The sample was serially diluted using 9ml of sterile distilled water. The diluted sample was inoculated on the agar medium by pour plate technique and incubated for 24-48 hours. Petriplates having countable colonies ranging from 30-300 are chosen for enumeration and counted.

DNA Extraction, PCR Amplification and Gene Sequencing

Extraction of genomic DNA of the bacterial isolates was done according to the method of Ausubel *et al*.[15] Amplification of gene-encoding small subunit rRNA was carried out using eubacterial 16S rRNA primers [forward primer 5'-AGAGTTTGATCCTGGCTCAG-3' (E. Coli positions 8-27) and reverse primer 5'-ACGGCTACCTTGTTACGACTT-3' (E. Coli positions 1490 – 1509); Weisburg *et al*.[16]]. Polymerase chain reaction (PCR) was performed with 50µL of a reaction mixture containing 2 µL (10 ng) of DNA as the template, each primer at a concentration of 0.5µM, 1.5 mM $MgCl_2$, and each deoxynucleoside triphosphate (dNTP) at a concentration of 50µM, as well as 1µL of Taq DNA polymerase and buffer. A mastercycler personal instrument was used for carrying out PCR with the following program: initial denaturation at 95°C for 5 min; 35 cycles of denaturation (30 sec at 95°C), annealing (1min at 50°C), and extension (2 min at 72°C); followed by a final extension (at 72°C for 10 min). The amplified product was purified, cloned and DNA sequencing for all the isolates were carried out with isolated plasmids from the clones.

Mass Loss Measurements

Commercially available aluminium and copper metal sheets (composition in Table 1) were machined into coupons (7.5 x 1.9 x 0.3cm) as per ASTM G184[(1)]. The metal coupons were polished with 200,400,600 and 800 grit emery paper and then degreased using trichloroethylene, and dried.

TABLE 1

Composition of metals

Elements	% Composition	
	Aluminium	Copper
Si	0.16	0.058
Fe	0.56	0.0005
Cu	0.064	95.16
Mn	0.006	0.002
Mg	0.0007	0.00007
Zn	0.003	2.78
Ti	0.014	-
Cr	0.002	-
Ni	0.01	0.012
Pb	0.017	0.032
Sn	0.003	1.31
Na	0.0007	-
Ca	0.006	-
B	0.006	-
Zr	0.002	-
V	0.015	-
Be	0.00004	-
Sr	0.0002	-
Co	0.011	-
Cd	0.0004	-
Sb	0.002	0.34
Ga	0.009	-
P	0.002	0.027
Li	0.00001	-
Al	99.03	0.16
S	-	0.029
Bi	-	0.006
As	-	0.068
Ag	-	0.004

Jatropha curcas biodiesel (JBD) was purchased from a biodiesel exporter in India and commercial diesel was purchased from a nearby petrol bunk.

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(1) American Society for Testing and Materials International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA, 19428 – 2959, USA.

The following five fuel mixtures were used as test media.

- CD - 100% commercial diesel
- B5 - 5% JBD and 95% CD (% by volume)
- B10 - 10% JBD and 90% CD (% by volume)
- B20 - 20% JBD and 80% CD (% by volume)
- B100 - 100% JBD

Control System: 800ml fuel mixture + 2% (v/v) water (500ppm chloride) to simulate corrosion conditions.

Experimental System: 800ml fuel mixture + 2% (v/v) water (500ppm chloride) + 0.5% (v/v) of bacterial inoculums (a load of 1 x 10^6 Colony Forming Units/ml)

The mass loss measurement was carried out as per ASTM G1 standard. Previously weighted metal coupons were immersed in the test matrices and agitated using magnetic stirrer. After 100h, the coupons were removed and pickled in pickling solutions (ASTM G3), washed with water and dried. Final masses of the coupons in each system were taken and the mean corrosion rates (in triplicates) were calculated and expressed in mpy. The corrosion rate was calculated using the following formula.

$$\text{Corrosion Rate (mpy)} = \frac{3.45\times10^{6}\times mass\ loss(gram)}{Density\left(g/cm^{2}\right)\times Area\left(cm^{2}\right)\times Time(hour)} \qquad (1)$$

Surface morphology.

Scanning Electron Microscopy (SEM)

For SEM analysis, the surface of the coupons exposed to the various test matrices for 100 hours was exposed to 2.5% gluteraldehyde for 8 hours and subsequently washed with a graded series (30%, 50%, 70% and 100%) of ethanol for dehydration. The sample was then coated with gold alloy prior to SEM observations. The entire surface area of the coupon was examined to locate sessile bacteria.

Laser Profilometry

A 3D optical profiler was used to map the surface topography and determine the pit distributions and depth for aluminum, and copper samples immersed in the control and experimental systems for 100 hours., 35 x 12 mm^2 areas of both faces (called inside and outside by convention) were examined by taking height measurements at 10µm intervals in both the longitudinal and transverse direction. A peak count operation was performed to determine the pit depths and densities. During laser profilometry analysis, the highest point on the specimen was assumed to correspond to the original metal surface (prior to immersion) and the pit depths are reported relative to this point.

RESULTS AND DISCUSSION

Identification of bacteria

Preliminary identification of three bactiral strains indicated that the isolates belonged to the genus *Bacillus* sp. DNA extraction and amplification of targeting bacterial 16S rRNA gene was performed using eubacterial 16S rRNA primers.The 16S rRNA gene were cloned and the isolated plasmids from the clones were subjected to 16S rRNA gene sequencing.The sequences obtained were matched with the previously published sequences available in NCBI[(2)] (National Centre for Biotechnology Information) using BLAST. Multiple sequence alignments were carried out with a collection of taxonomically related sequences. Sequence alignment and comparison revealed similarity with ***Bacillus pumilus*** (Figure 1). The nucleotide sequence data have been deposited in GenBank[†] under the accession numbers **KF410588, KF410589** and **KF410590.**

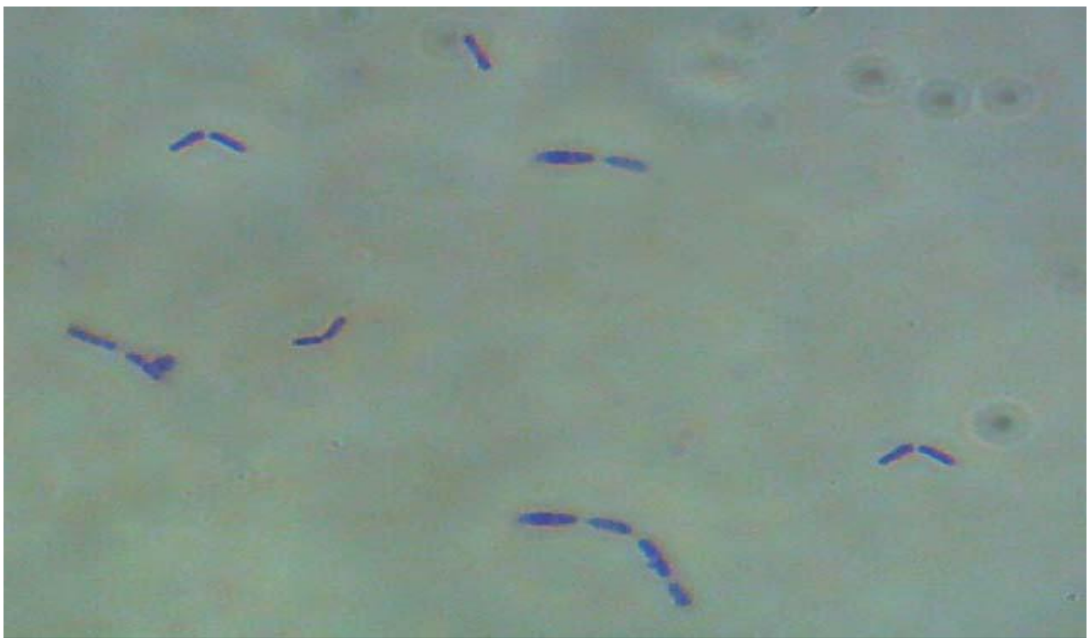


Figure 1: Microscopic image of *Bacillus pumilus* obtained from the JBD sediment

Mass loss measurements:

Aluminium

The mean corrosion rates of aluminium in JBD and its various blends with CD for the control and experimental systems as measured by mass loss measurements are given in Table 2

(2) National Centre for Biotechnology Information, U.S.National Library of Medicine, 8600 Rockville Pike, Bethesda MD, 20894 USA.

† Trademark

Table 2
Mean corrosion rates of aluminium in B100 and CD blends

Fuel system	Corrosion rate (mpy)				
	CD	**B5**	**B10**	**B20**	**B100**
Control System	0.24 ± 0.0190	0.25 ± 0.1029	0.20 ± 0.0273	0.18 ± 0.0077	0.59 ± 0.1185
Experimental System	0.26 ± 0.0280	0.17 ± 0.0615	0.16 ± 0.0279	0.21 ± 0.0889	0.96 ± 0.4991
Ratio of corrosion rate in experimental / control system	1.08	0.68	0.80	1.17	1.63

Biodiesel is more corrosive than petrodiesel which may be due to the presence of free fatty acids. This is reflected in Table 2 where the corrosion rates of aluminium are the highest in B100 both in the control and experimental systems. Comparison of ratio of corrosion rates in experimental/control system reveals that *Bacillus pumilus* has a diminishing effect on the corrosion rate of aluminium in B5 and B10 blends whereas it has an accelerating effect in B20 and B100 test media.

Copper

The mean corrosion rates of copper in JBD and its various blends with CD for the control and experimental systems as measured by mass loss measurements are given in Table 3.

Table 3
Mean corrosion rates of copper in B100 and CD blends

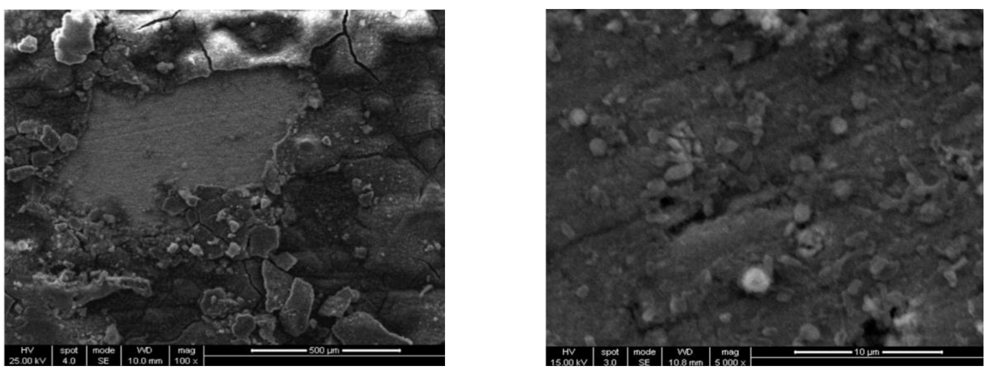
Fuel system	Corrosion rate (mpy)				
	CD	**B5**	**B10**	**B20**	**B100**
Control System	0.19 ± 0.0298	0.16 ± 0.0645	0.39 ± 0.0649	0.48 ± 0.0469	1.06 ± 0.0891
Experimental System	0.21 ± 0.0205	0.14 ± 0.0138	0.17 ± 0.0128	0.23 ± 0.0179	1.57 ± 0.2222
Ratio of corrosion rate in experimental / control system	1.11	0.88	0.44	0.48	1.48

The corrosion rate of copper is found to be the highest in B100 in the control system. In both the systems the corrosiveness of JBD increases with increasing biodiesel content. It is also evident from Table 3 that the presence of Bacillus *pumilus* in JBD has decreased the corrosion of copper in B5, B10 and B20 blends, which may be due to the formation of biofilm.

Several studies have been reported on the corrosivity of biodiesels on various metals and their alloys. The effect of different biodiesels produced from nonedible oils on piston metal and liner was investigated by Kaul *et al*.[17] and the results showed that biodiesels obtained from salvadora and jatropha were found to be more damaging on the metals. Geller *et al*.[18] found that copper alloys are more prone to corrosion in the presence of fat-based biodiesels. In a couple of studies conducted on the corrosion of copper and aluminum in the presence of rapeseed[19] and sunflower[20] biodiesels, copper was found to be more vulnerable to corrosion.

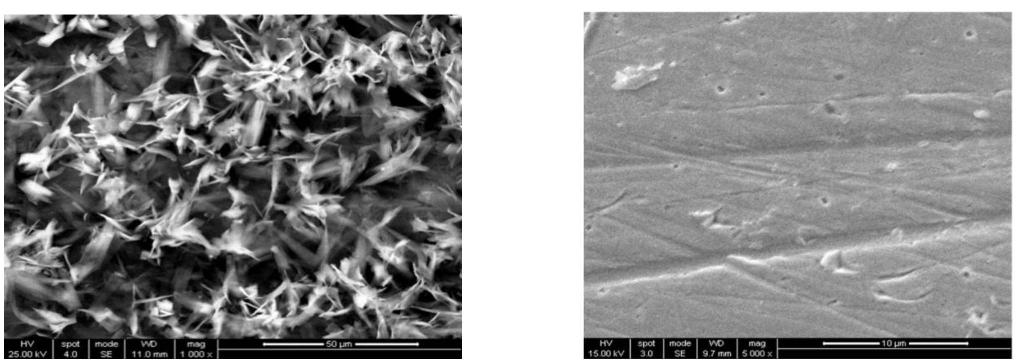
Scanning Electron Microscopy

The SEM micrographs of aluminium in the experimental system in B100 (Figure 2a) and CD (Figure 2b) and of copper in B100 (Figure 3a) and CD (Figure 3b) without removal of corrosion products on the metal surface are shown below. It is very clear that the coupons are covered with corrosion products. Aluminium exposed to B100 in the experimental system shows cracks in the corrosion products formed on the metal surface. For copper in B100 the surface is covered by the corrosion product in crystalline form which is not obviously seen in CD.



(a) (b)

Figure 2:SEM images of aluminium (a) JBD and (b) CD in the experimental system



(a) (b)

Figure 3: SEM images of copper (a) JBD and (b) CD in the experimental system

Laser profilometry
Laser profilometry is used to determine the surface topography of corroded materials without altering the sample surface through physical contact.

Surface profiles (3D), pit distributions and roughness parameters of aluminium and copper in both the control and experimental systems in B100 are described in this section

<u>Aluminium</u>
The surface profile and the pit distribution histogram of aluminium in B100 for the control system are presented in Figure 4 and Figure 5 and that for the experimental system in Figure 6 and Figure 7 respectively.

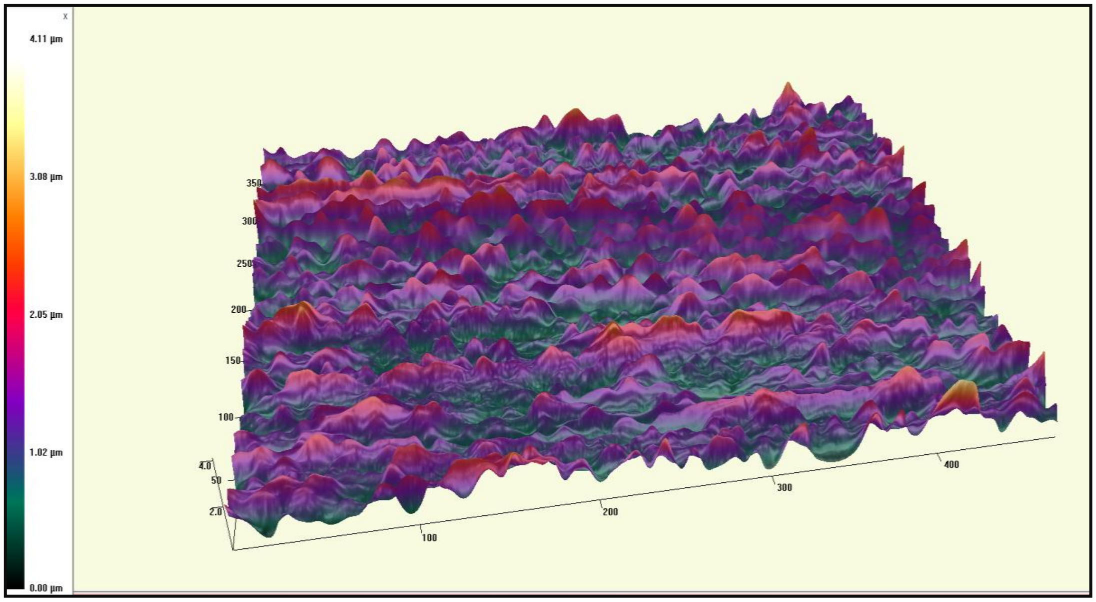


Figure 4: Surface profile (3D image) of aluminium in control system (B100)

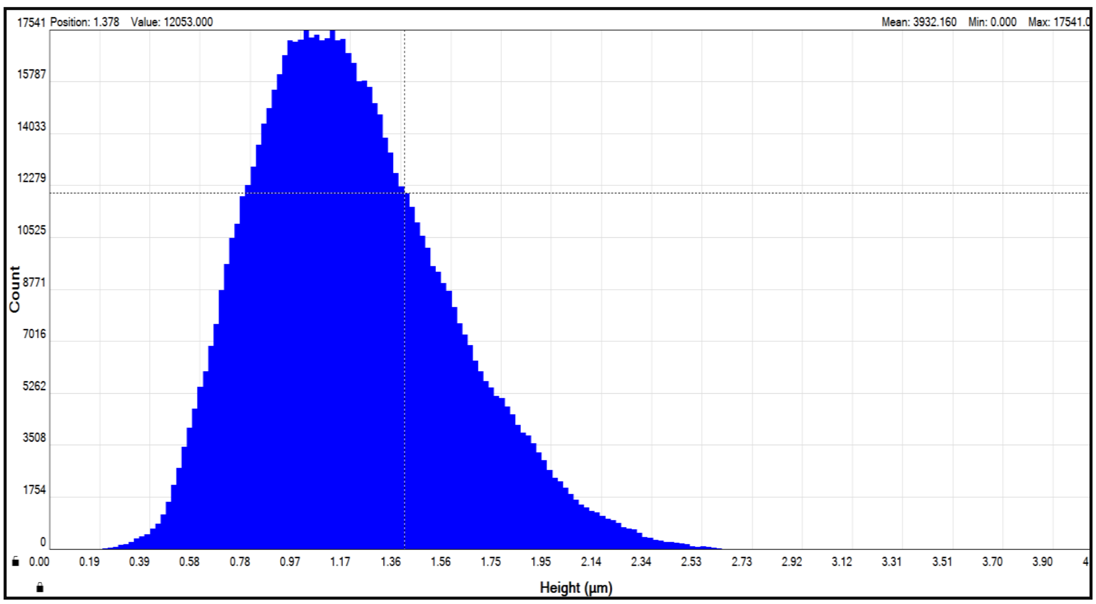


Figure 5: Pit distribution histogram of aluminium in control system (B100)

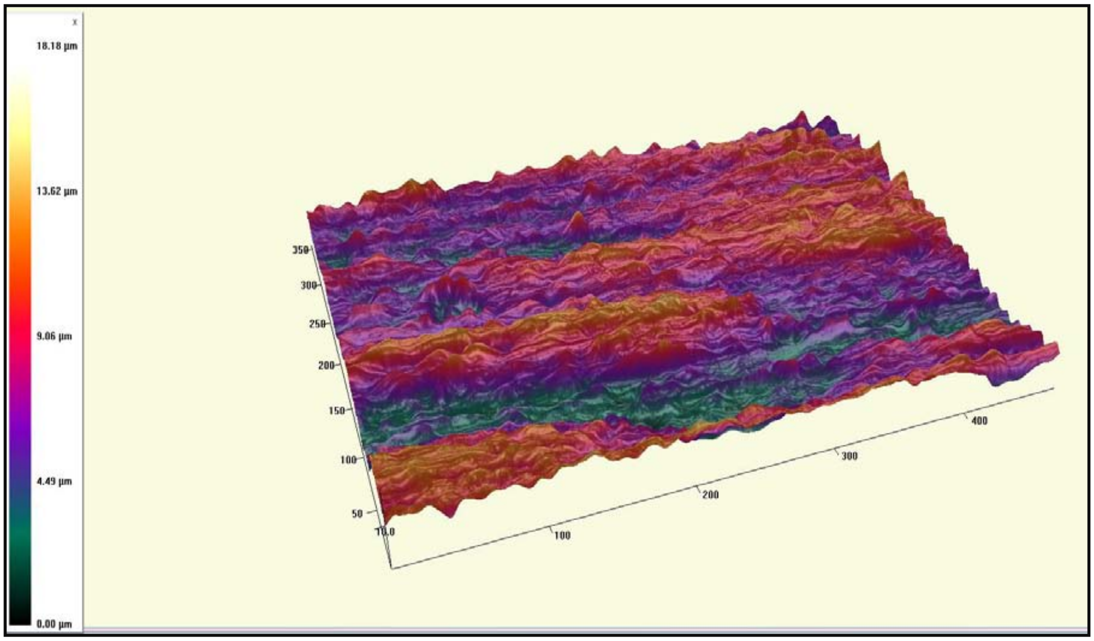


Figure 6: **Surface profile (3D image) of aluminium in experimental system (B100)**

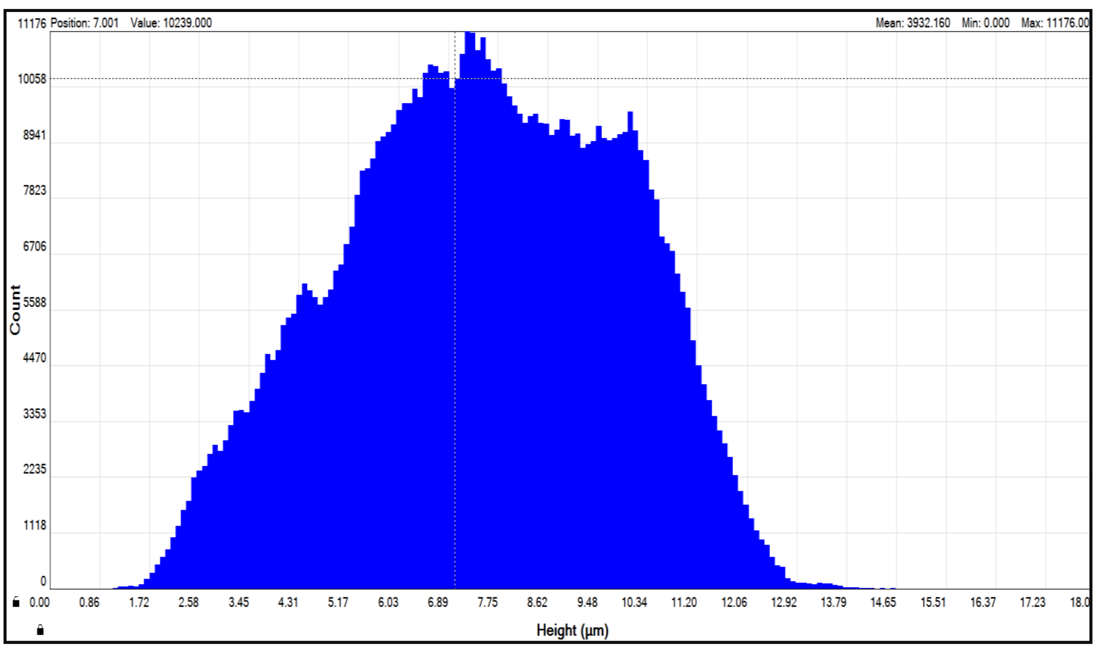


Figure 7: Pit distribution histogram of the aluminium in experimental system (B100)

Copper

The surface profile and the pit distribution histogram of copper in B100 for the control system are presented in Figure 8 and Figure 9 and that for the experimental system in Figure 10 and Figure 11 respectively.

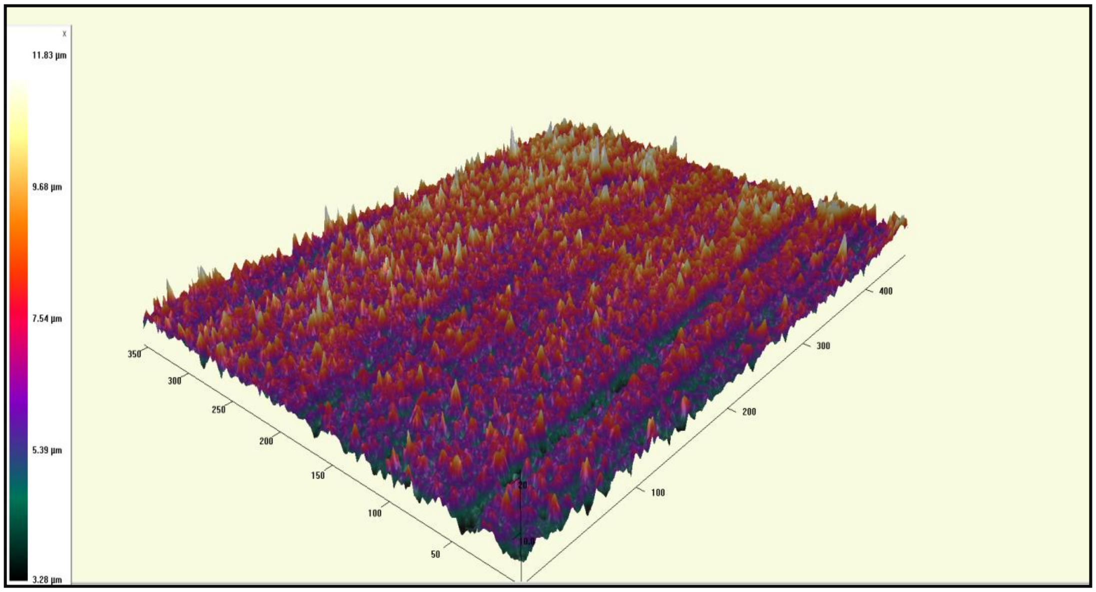


Figure 8: Surface profile (3D image) of copper in control system (B100)

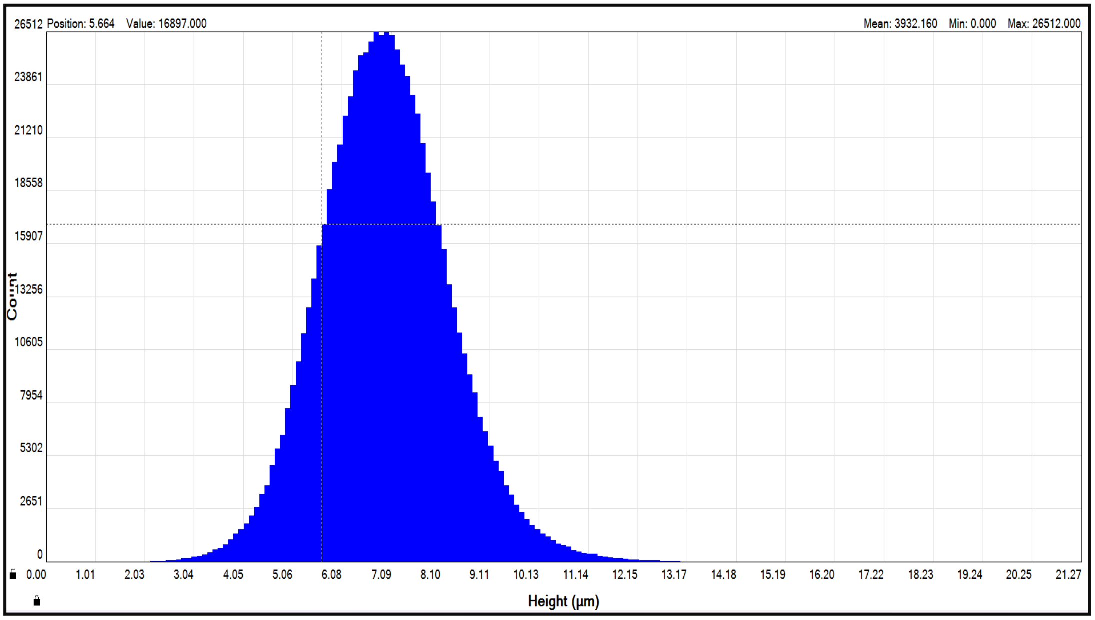


Figure 9: Pit distribution histogram of copper in control system (B100)

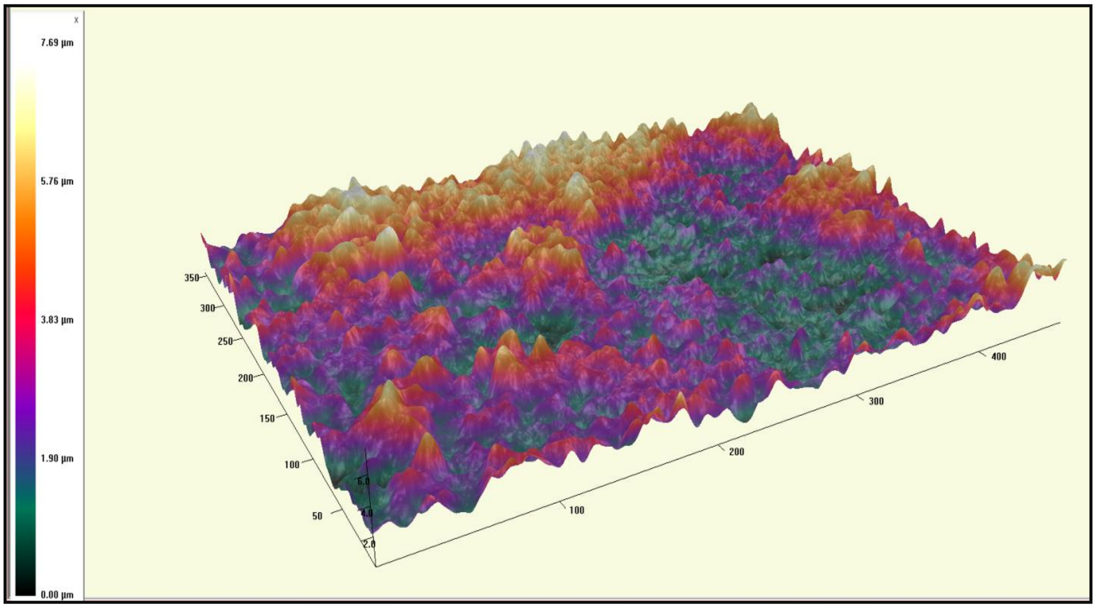


Figure 10: Surface profile (3D image) of copper in experimental system (B100)

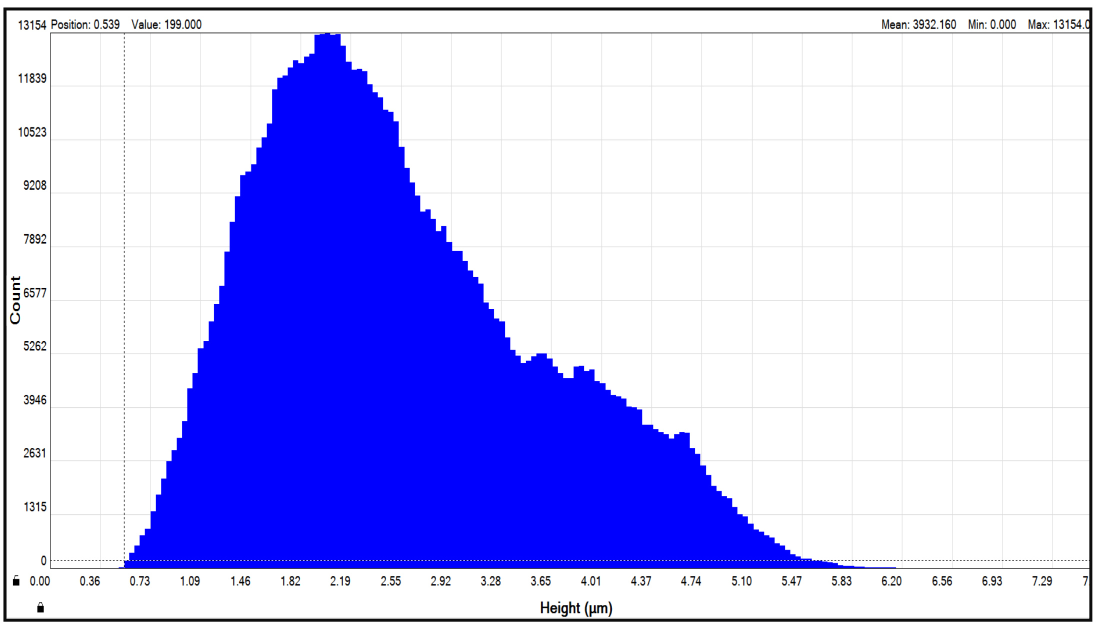


Figure 11: Pit distribution histogram of copper in experimental system (B100)

Roughness parameters

Corrosion is a surface phenomenon and hence surface roughness which is the measure of texture of a surface is significant in corrosion studies. It is quantified by the vertical deviations of a real surface from its ideal form. If these deviations are large, the surface is rough; if they are small the surface is smooth. The roughness indicates how the real object will interact with the environment and hence often predicts the corrosion of metal surfaces. The roughness value is either calculated on a profile (line) or on a surface (area). But the profile roughness parameters are more common. Of the many different roughness parameters in use the most common parameters are R_a, R_q and R_t. These parameters are of statistical nature.

R_a is the arithmetic average of the roughness profile.

R_q also known as **R_{rms}** is the root mean square average between the height deviations and the mean line taken over the evaluation length. It describes the finish of optical surfaces.

R_t represents the maximum height – the vertical distance between the highest and lowest point. It describes the overall roughness of the surface.

The profilometric mean roughness parameters R_a, R_q and R_t of the surface profile of the metals taken at different positions on the metal surface, and the mean and the standard deviation were calculated are tabulated in Table 4 for aluminium and Table 5 for copper.

Table 4
Roughness parameters for aluminium (B100)

	Control			Experimental		
	Ra (µm)	**Rq (µm)**	**Rt (µm)**	**Ra (µm)**	**Rq (µm)**	**Rt (µm)**
I	0.2642	0.3263	1.709	0.9160	1.092	5.098
II	0.2065	0.2533	1.174	1.517	1.905	7.564
III	0.3298	0.3954	1.618	1.171	1.471	5.877
Mean	**0.2668**	**0.3250**	**1.500**	**1.201**	**1.490**	**6.180**
SD	**0.0504**	**0.0580**	**0.2335**	**0.2465**	**0.3322**	**1.029**

Table 5
Roughness parameters for copper (B100)

	Control			Experimental		
	Ra (µm)	**Rq (µm)**	**Rt (µm)**	**Ra (µm)**	**Rq (µm)**	**Rt (µm)**
I	0.8473	1.077	5.975	0.5568	0.7336	3.566
II	0.6659	0.8744	4.975	0.3483	0.4420	2.603
III	0.7842	0.9920	5.423	0.6728	0.8491	4.215
Mean	**0.7658**	**0.9810**	**5.457**	**0.5260**	**0.6749**	**3.461**
SD	**0.0752**	**0.0829**	**0.4090**	**0.1343**	**0.1713**	**0.6623**

For aluminium the roughness parameters are found to be greater in the presence of bacteria than in its absence while the opposite trend is noticed for copper.

Similar observation can also be inferred in the pit distribution diagrams.The data are presented in Table 6

Table 6
Pit distribution data

Metal	Control		Experimental	
	Maximum pit counts	Position (μm)	Maximum pit counts	Position (μm)
Aluminium	12,053	1.378	10,239	7.001
Copper	16,897	5.664	199	0.539

No correlation could be established between laser profile data with mass loss results since mass loss describes the general corrosion while laser profile details localized corrosion.

CONCLUSION

- Of all the test matrices used, both aluminium and copper exhibit the highest corrosion rates in B100 in the control as well in the experimental systems.
- For aluminium the corrosion rates in B5 and B10 blends have decreased in the presence of *Bacillus pumilus*.
- For copper, increase in biodiesel content enhances the corrosiveness of *Jatropha curcas* biodiesel in the absence and presence of *Bacillus pumilus*.
- While comparing the corrosion rates of copper in the presence and absence of *Bacillus pumilus* it is observed that the corrosion rates in B5, B10 and B20 blends are lesser in the presence of bacteria than that in the absence of bacteria.

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