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Research Article

Microbiologically Influenced Corrosion of Carbon Steel Exposed to Biodiesel

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Environmental concerns over worsening air pollution problems caused by emissions from vehicles and depletion of fossil fuels have forced us to seek fuels such as biodiesel which can supplement petrofuels. Biodiesels have the ability to retain water and provide a conducive environment for microbiologically influenced corrosion (MIC) which may cause difficulties during transportation, storage, and their use. This paper analyses the influence of bacteria on the corrosivity of biodiesel obtained from *Jatropha curcas* on carbon steel using mass loss method. Carbon steel showed the highest corrosion rates in B100 (100% biodiesel) both in the presence and in absence of bacteria. The surface analysis of the metal was carried out using SEM.

1. Introduction

The alarm over fossil fuel depletion, greenhouse gas emissions, and energy security has necessitated the development of alternative renewable energy sources for which biodiesels hold great promise [1]. Besides having a positive energy balance, use of biodiesel in conventional diesel engine results in substantial reduction of unburned hydrocarbons, carbon monoxide, and particulate matters. Nowadays all the countries in the world including those with surplus energy are banking upon biodiesel as an alternative energy source. India has also realized the enormous potential for biodiesel and increased its emphasis on biodiesel production. Under Indian conditions, two nonedible plants *Jatropha curcas* and *Pongamia pinnata* are widely used for the production of biodiesel. Of the two, jatropha has been identified as the most suitable one because it is a plant of tropics with high oil content and it can withstand extreme drought conditions [2, 3].

In spite of the numerous advantages that the biodiesel possesses it has its share of drawbacks. Corrosion is one of the consequences of biodiesel compatibility issues [4]. Biodiesels may form sediments or cause corrosion when they come into contact with construction materials such as carbon steel, stainless steel, or aluminium which are used for making storage tanks, pipes, and pumping equipment [5]. Recent research has shown that biodiesel can accelerate corrosion of carbon steel used in manufacturing pipelines, storage tanks, and other components of fuel infrastructure. Hence there is an increasing need for research for material compatibility with biodiesel for its judicial application.

Biodiesels are fatty acid methyl esters produced from vegetable oils by a process known as transesterification. Biodiesel undergoes degradation through moisture absorption, oxidation, and attack by microorganisms during storage or use and becomes more corrosive. Both petroleum diesel and biodiesel are often contaminated with microorganisms. Water is one of the essential components for microbial growth to occur. Water enters into the fuel system during production, transportation, and storage. It is very hard to remove water from fuel systems especially when blended with biodiesel

Table 1: Elemental composition of carbon steel.

Element	Fe	C	Mn	Zn	P	Al	S	Si	Others
% composition	99.22	0.15	0.31	0.002	0.002	0.025	0.002	0.018	0.271

since biodiesels are more hygroscopic than petroleum diesel. While investigating the corrosion problems in petroleum product pipeline, Maruthamuthu et al. [6] reported 2–11% water contamination which also contained chloride ions. The corrosivity of two samples of biodiesels on mild steel was studied by Meenakshi et al. [7] in the presence of 3% NaCl solution to depict water contamination. Aktas et al. [8] reported that water present in the biodiesel contained ions like chloride and/or sulphide and caused pitting corrosion of metals. The water condenses and collects at the bottom of the fuel tanks or pipes and causes microbial growth leading to the formation of sediments, sludge, and slime resulting in fuel deterioration and corrosion which normally occurs under the resulting biomass [9]. Microbiologically influenced corrosion (MIC) is a serious problem in biodiesel handling facilities. Few works are available on this topic in the literature.

A pioneering work using wire beam electrodes technique carried out by Wang et al. [10] reported that corrosion of carbon steel occurred at the surface exposed to water while the cathodes were formed at water-biodiesel interfaces. Lee et al. [11] observed biofouling while evaluating MIC of metal and alloys in biodiesel, ultralow sulphur diesel, and their blends. Klofutar and Golob [12] reported that absence of water was one of the significant criteria for the prevention of microorganisms in fuels. Biodiesel blends showed an increased bacterial growth and activity compared to neat diesel when diesel-biodiesel blends were incubated with contaminated inoculation water collected from diesel storage tanks [13]. Hence knowledge about the nature of microbes that survive in biodiesel and the ingredients that help in their growth will help us control MIC and the present work aims to investigate the influence of bacteria isolated from the sediments of stored biodiesel obtained from *Jatropha curcas* on the corrosivity of *Jatropha curcas* biodiesel and its blends on carbon steel.

2. Materials and Methods

2.1. Sample Collection, Enumeration, and Isolation of Bacteria. The deposit at the bottom of the container having a two-year-old sample of *Jatropha curcas* biodiesel was collected in a sterile container. The collected sample was serially diluted (10-fold) using sterile distilled water. This is then inoculated on the agar medium by pour plate technique and incubated for 24–48 hours. Petri plates having countable colonies ranging from 30 to 300 are chosen for enumeration and counted (3 strains).

2.2. DNA Extraction, PCR Amplification, and Gene Sequencing. The molecular identification of bacterial isolates was done by 16S rRNA sequencing. Genomic DNA were isolated from the bacterial strains, amplified by polymerase chain reaction (PCR) using universal 16S rRNA primers, cloned, and sequenced with dideoxy nucleotide. The sequences obtained were matched with the previously published sequences available in National Centre for Biological Information (NCBI) using BLAST.

2.3. Corrosion Rate Determination by Mass Loss Method. Commercially available carbon steel sheet was machined into coupons of size $7.5 \times 1.9 \times 0.3$ cm as per ASTM G184 and holes were drilled on the top centre of the coupons. The elemental composition of carbon steel is given in Table 1. *Jatropha curcas* biodiesel (JBD) was purchased from a biodiesel exporter in India and commercial diesel (CD) was purchased from a nearby petrol bunk. The carbon steel coupons were polished with 400, 600, and 800 grit emery paper and then degreased using trichloroethylene.

The following four fuel matrices were used as test media:

(i) CD: 100% commercial diesel,

(ii) B5: 5% JBD and 95% CD (% by volume),

(iii) B20: 20% JBD and 80% CD (% by volume),

(iv) B100: 100% JBD.

The experiment was carried out in the absence (control system) and presence (experimental system) of bacteria. The control system consisted of 800 mL fuel mixture with 2% (v/v) water (500 ppm chloride) to simulate corrosion conditions while the experimental system used was 800 mL fuel mixture with 2% (v/v) water (500 ppm chloride) and 0.5% (v/v) of bacterial inoculum (a load of 1×10^6 CFU/mL).

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

$$\text{Corrosion Rate (mpy)} = \frac{3.45 \times 10^6 \times \text{mass loss (gram)}}{\text{Density (g/cm}^2) \times \text{Area (cm}^2) \times \text{Time (hour)}}. \quad (1)$$

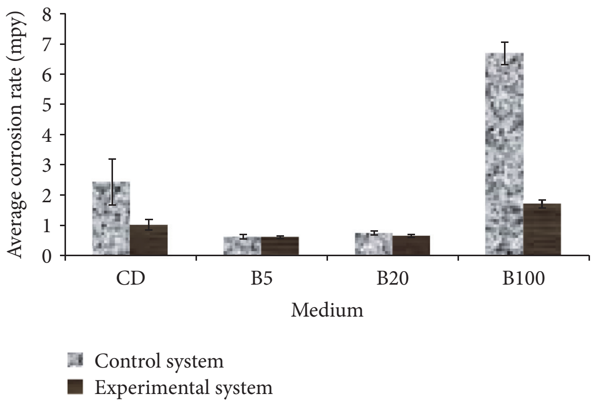


Figure 1: Corrosion rates of carbon steel in the control and experimental system.

2.4. Surface Analysis by Scanning Electron Microscopy (SEM). For SEM studies, the surface of the coupons exposed to CD and B100 test matrices for 100 h were chosen. The coupons were exposed to 2.5% glutaraldehyde for 8 h and subsequently washed with a graded series (30%, 50%, and 70% and 100%) of ethanol for dehydration. The samples were then coated with a gold alloy prior to SEM observations. The entire surface area of the coupon was examined to locate sessile bacteria.

3. Results and Discussion

3.1. Isolation of Bacteria. Three bacterial strains were isolated from the sediments formed in a two-year-old jatropha biodiesel left over from a previous research. Preliminary identification of the three bacterial strains obtained indicated that the isolates belonged to the genus *Bacillus* sp.

3.2. 16S rRNA Gene Sequence Analysis. Amplification of targeting bacterial 16S rRNA gene was performed using eubacterial 16S rRNA primers. The 16S rRNA gene was 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 (National Centre for Biological Information) using BLAST. Sequence alignment and comparison revealed similarity with *Bacillus pumilus*. The nucleotide sequence data have been deposited in GenBank under the accession numbers *KF410588*, *KF410589,* and *KF410590* [14]. *Bacillus* species constitute a diverse group of bacteria widely distributed in soil and aquatic environment. *Bacillus pumilus* is a gram-positive, aerobic, rod-shaped, endospore forming bacteria, belonging to the genus *Bacillus*. *Bacillus pumilus* resides in soils and some colonise in the root area of some plants.

3.3. Mass Loss Measurement. The corrosion rates of carbon steel in four test matrices in the control and experimental systems as determined by mass loss method are depicted in Figure 1. It is clear that carbon steel shows the highest corrosion rates in B100 both in the control and in experimental systems followed by CD, B5, and B20. The corrosion rates of carbon steel in B100 in the control and experimental systems are 6.69 ± 0.3731 mpy and 1.70 ± 0.1386 mpy, respectively. Several studies have shown that corrosion of metals in biodiesel is higher than that in petrodiesel and this may be due to the presence of water content, free fatty acids, and unconverted monoalkyl esters. [5, 15, 16]. It is also noted that the corrosion rate increases with increasing concentration of biodiesel in the blends. The corrosivity of biodiesel blends is found to be lesser compared to that of petrodiesel alone. The same trend was observed by Ambrozin et al. [17]. Also the corrosion rates of carbon steel in all the four fuel matrices used in this study were found to be lesser in the presence of *Bacillus pumilus* compared to the control system and a marked difference is noticed in the corrosion rates of carbon steel in B100 between the control and experimental systems. This may be due to the formation of a protective film/biofilm on the surface of carbon steel where biological activities modify the environmental conditions at the metal/solution interface which may result in the reduction of corrosion rates in the presence of bacteria [18]. Studies have shown that various strains of bacteria like *Staphylococcus Sp.* and *Pseudomonas cichorii* formed corrosion inhibiting biofilm on mild steel [19, 20]. A significant reduction in corrosion rates of brass was achieved by *Bacillus subtilis* bacterial biofilm [21].

Corrosion studies involving *Bacillus pumilus* are limited. Bolton et al. [22] investigated the role of *Bacillus pumilus* isolated from samples taken from corroding galvanised steel pipes conveying water on the corrosion of steel and galvanised steel. The results showed that *Bacillus pumilus* had increased the corrosion of zinc galvanised steel but did not increase the corrosion rate of steel.

3.4. Surface Analysis by Scanning Electron Microscopy. Figure 2 shows the SEM micrographs of carbon steel in CD and B100 in the experimental system after exposure to the bacterial system, without removal of corrosion products on the metal surface. It shows that the coupons are covered with dense and lumpy corrosion products.

4. Conclusion

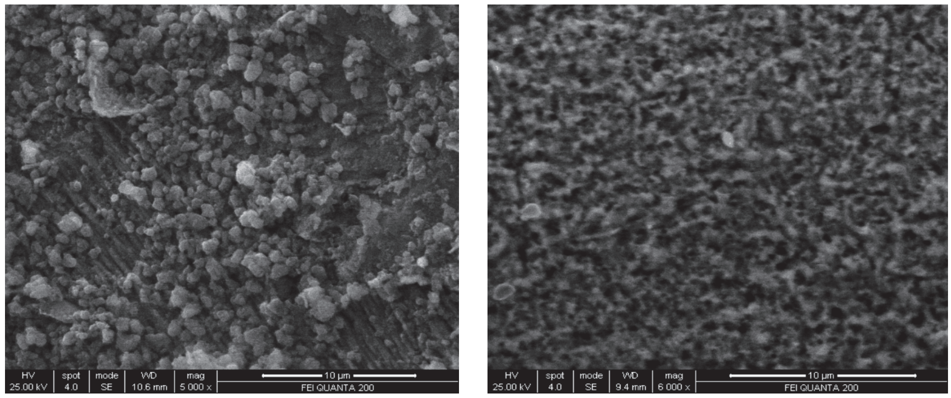
(i) Carbon steel shows the highest corrosion rates in B100 in both the control and experimental systems.

(ii) The corrosion rates of carbon steel increase with increasing concentration of biodiesel in the blends.

(iii) In the presence of *Bacillus pumilus* carbon steel shows lesser corrosion than that in the control system in all the test matrices.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this article.



(a) (b)

Figure 2: SEM images of carbon steel (a) CD and (b) B100 in the experimental system.

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