2

REVIEW OF LITERATURE

Rapid growth in population coupled with industrial and technological developments is leading towards the depletion of limited fossil fuel resources of the world and biodiesel, a promising, clean and ecofriendly fuel is catching world's attention as alternative fuel. Implementation of very stringent emission standards in the near future has made us look for alternative fuel resources. Typical oils used for biodiesel production are rapeseed, canola, sunflower, soyabean, palm oil, etc. Used vegetable oils from fast food restaurants and food processing facilities are also being considered as potential source of biodiesel.

As the alternative fuel industry expands, there is a need for research to bring out the desirable benefits of alternative fuels. Material compatibility is a major concern with biodiesel usage, since biodiesel degrades through moisture absorption, oxidation and attack by microorganisms, thereby becoming more corrosive.

Besides a brief description of synthesis and standardisation of biodiesel, this chapter explains the material compatibility in biodiesel, particularly in the microbial environment. Since the identification of microorganisms responsible for MIC is of atmost importance, the molecular techniques used for it are discussed here.

2.1 *Jatropha curcas*

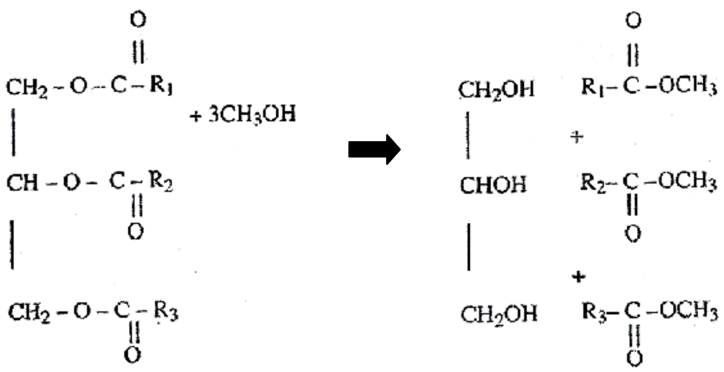
Jatropha curcas, the wonder plant of the era is from South America. Jatropha has more than 137 species out of which about 12 are seen in different parts of South East Asia. The most important and the commercially viable out of these is ***Jatropha curcas*** because of its higher oil content. Jatropha has assumed tremendous importance and potential as a non edible oil seed producing plant especially for production of biodiesel throughout the world. Jatropha oil is one of the best sources for production of biodiesel - an eco friendly fuel- a replacement for mineral diesel.Jatropha is a poorman's fuel and therefore a farmer's delight. Plantation of jatropha, therefore, will make our farmers and villagers self sufficient once they cultivate jatropha in their land. Moreover, as jatropha grows in waste land as well, we can now use our non agricultural land in cultivating jatropha which will be a boon to our national economy.

Jatropha curcas is a promising feedstock for biodiesel production as it does not compete with food sources. Conventional production of biodiesel from *J. curcas* seeds involve two main processing steps; extraction of oil and subsequent esterification / transesterification to fatty acid methyl esters (FAME).

Figure 1. *Jatropha curcas* Tree

2.2 Synthesis of Biodiesel

Biodiesel is prepared by transesterifying the vegetable oils with methanol at 60-70°C in the presence of KOH as catalyst. The overall transesterification reaction is



After separation of glycerol, biodiesel is purified by washing with hot water (70°C) and finally dried using molecular sieves.

2.3 Corrosivity of biodiesel

Even though the use of biodiesel in automobile can significantly reduce our dependence on fossil fuels and help reduce environmental pollution, there are concerns over the compatibility of currently used automotive materials in biodiesel. Works done so far on the compatibility of biodiesel with automotive materials suggest that because of certain inherent characteristics of biodiesel, it varies distinctly from petrodiesel and causes a number of operational problems including material incompatibility **(Haseeb *et al.* 2011)**. Biodiesel and biodiesel blends form sediments when in contact with metals like carbon steel, stainless steel or aluminium which are used for making tankers, storage tanks, pipes and pumping equipment.

2.4 Corrosion of metals

Corrosion according to ISO 8044 standard, is defined as "physicochemical interaction (usually of an electrochemical nature) between a metal and its environment which results in changes in the properties of the metal and which may often lead to impairment of the function of the metal, the environment, or the technical system of which these form a part" (**Javaherdashti 2008; Mattson 1989**). In a sense corrosion can be viewed as 'extractive metallurgy in reverse' **(Fontana 1986).**

2.5 Microbiologically Influenced Corrosion (MIC)

MIC is not a new corrosion mechanism; rather it integrates the role of microorganisms on the kinetics of corrosion processes of metals. MIC is defined as an electrochemical type of corrosion in which certain organisms have role either enhancing or inhibiting. MIC can occur in almost all environments - solid, fresh water, sea water and all industries like oil and gas, power generation and marine industries. Different estimations from various industrial sectors indicate that MIC is costly.Investigations about the role of micro organisms in corrosion began only in the late 19th century.

2.6 Classification of bacteria

The factors enabling microbiologists to differentiate the different types of bacteria from one another are: (**Javaherdashti 2008; Geesey 1993)**

Shape and appearance

1. Vibrio: Comma-shaped cells
2. Bacillus: rod-shaped cells
3. Coccus: round-shaped cells
4. Myces for filamentous fungi- like cells, etc.

Temperature

1 .Mesophile: bacteria that grow best at 20-35^{0}C

2. Thermophile: bacteria active above 40^{0}C

Oxygen consumption

1. Strict or obligate anaerobes which will not function in the presence of oxygen
2. Aerobes which require oxygen in their metabolism
3. Facultative anaerobes which can function in either the absence or presence of oxygen
4. Micro-aerophiles which use low levels of oxygen
5. Aero-tolerants - anaerobes not affected by the presence of oxygen

Beech and Gaylarde (1999) classified the organisms responsible for corrosion failures of materials into different groups like metal - depositing bacteria (MDB), metal - reducing bacteria(MRB),slime-producing bacteria, acid - producing bacteria (APB), and sulphate reducing bacteria (SRB). These organisms coexist in naturally occurring biofilms often forming communities able to affect electrochemical processes through co-operative metabolism which individual species have difficulty to initiate.

SRB are anaerobic bacteria which derive their energy from organic nutrients. They do not require oxygen for growth and nativity; instead they use sulphate with the consequent production of sulphide. SRB affect almost all types of engineering materials: copper, aluminium, nickel, zinc, titanium and their alloys, mild steel and stainless steel. Generally SRB were considered to be the major bacteria involved in MIC and hence most of the research focused on SRB. However corrosion may also be due to aerobic bacteria and fungi; recent studies have shown that in diesel and naphtha pipelines corrosion took place in the absence of SRB.

2.7 *Bacillus pumilus*

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. *Bacillus pumilus* grows at pH 5.7-9.5, in 0-10% NaCl in the temperature range 5-15^{0}C to 40-50^{0}C

Maruthamuthu *et al*. (**2011**) investigated the corrosion problem in petroleum products transporting pipeline in South India. Even though a corrosion inhibitor was added, severe corrosion and microfouling problems occurred in the pipeline. Presence of different types of bacteria like heterotrophic bacteria, acid producers, iron bacteria, sulphate reducing bacteria and manganese oxidisers was reported in the products collected from different stations. In one of the stations, presence of manganese oxidiser (metal depositing bacteria), *Bacillus pumilus* was identified from the corrosion product.

MIC Issues of Biofuels

The chemical and microbiological compositions of hydrocarbon fuel have been studied for decade. MIC for crude and distillate fuels has been studied during all phases of exploration, transportation, storage and usage. Of the basic categories of fuels comprising of automotive gasoline, middle distillates (diesel and domestic heating oil) and aviation jet fuel, the group containing middle distillate is the most sensitive to the growth of microorganisms. The chemistries and microbiological constituents of alternative fuels are different from petroleum fuels. Biofuels particularly those of plant based methyl esters exhibit hygroscopic properties and are more prone to microbial spoilage.Water is essential for microbial growth to occur. Unfortunately it is very hard to exclude all water from fuel systems during daily operations, especially when biodiesels are added to the fuel mix.

2.8 Identification of bacteria

MIC is a multidisciplinary subject that integrates the fields of material science, chemistry, microbiology and biochemistry. Identification of microorganisms responsible for MIC is of atmost importance**.**

2.8.1 Molecular Techniques

Molecular techniques involving bacterial deoxynucleic acid (DNA) and ribonucleic acid (RNA) are now being applied to assess MIC. These tools can unravel the spatial distribution of the microbial communities and identify bacteria which could potentially influence MIC. Sequence variation in ribosomal RNA gene is exploited for inferring phylogenetic relationship among microorganisms.

2.8.2 Molecular chronometers -16S rRNA

At present, 16S rRNAs are the most useful and most used of the molecular chronometers. They show a high degree of functionality constancy, which assures relatively good clocklike behaviour **(Woese 1987).** They occur in all organisms, and different positions in their sequences change at very different rates, allowing most phylogenetic relationships (including the most distant) to be measured, which makes their range all-encompassing. Their sizes are large and they consist of many domains. There are about 50 helical stalks in the 16S rRNA secondary structure and roughly twice that number in the 23S rRNA (**Gutell *et al.* 1985; Olsen *et al.* 1986**), which makes them accurate chronometers on two counts.

2.8.3 Polymerase Chain Reaction – PCR

The most important development in the application of 16S rRNA sequences to bacterial systematics was the advent of PCR (Polymerase chain reaction), which enable the amplification of these sequences without necessarily culturing the microorganisms **(Wilson *et al.*, 1997)**.PCR based assays provide rapid, simple and sensitive detection of bacterial genes. But this method has its own drawbacks.

From the literature reviewed so far it is understood that

- Biodiesel is a viable and better alternative to fossil fuels
- Biodiesels are in general more corrosive than petrodiesel due to the presence of free fatty acids
- Presence of microbes in petrodiesel and biodiesel may lead to serious problems like product deterioration, sludge formation and corrosion of metallic materials.
- Being hygroscopic, biodiesel and biodiesel blends with petrodiesel are prone to even more microbial contamination and biocorrosion than petrodiesel.
- Several works have been reported on the MIC of metals by bacteria isolated from petroleum products.
- Much MIC research has been concentrated on the corrosivity of SRB.

Availability of limited current literature on the corrosivity of biodiesels in the presence of microbes necessitates the need for further research in this field. With this background, the present research work is focused on the isolation of bacteria from the sediment of stored Ja*tropha curcas* biodiesel and its influence on the corrosivity of jatropha biodiesel on few industrial metals.