JOURNAL OF CRITICAL REVIEWS

ISSN- 2394-5125 VOL 7, ISSUE 13, 2020

A STUDY ON CORROSION INHIBITION BY LACTOBACILLUS LACTIS DERIVED SURFACTANT ON THIOBACILLUS SP.

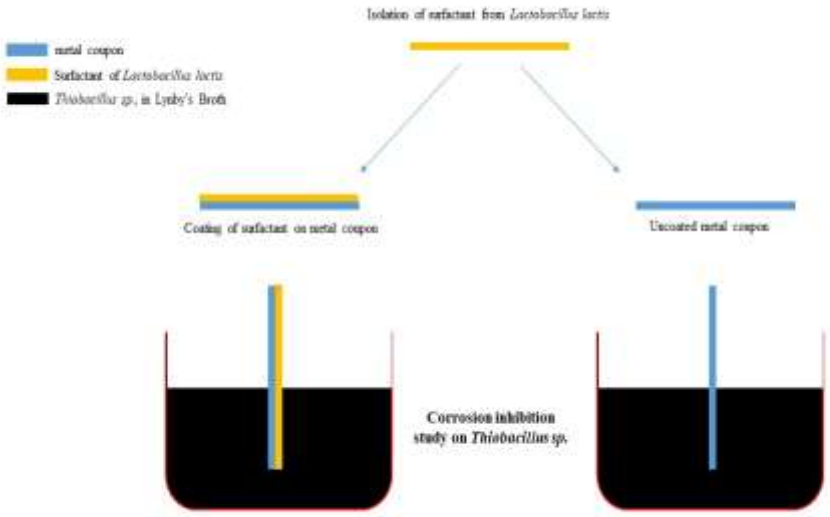
Challaraj Emmanuel E.S.[1], Rincy P. Benoy[2], Amjad Hassan Khan M K[3], Srinivasa Sundara Rajan R[4]

[1,2] Department of Life Sciences, Kristu Jayanti College (Autonomous), Bengaluru, Karnataka
[2]-Department of Electronics, Kristu Jayanti College (Autonomous), Bengaluru, Karnataka
[3] Research Scholar, CSIR - Central Electro Chemical Research Institute, Karaikudi, Tamil Nadu

Received: 14 March 2020 Revised and Accepted: 8 July 2020

ABSTRACT: Lactobacillus is a probiotic bacteria which produces biosurfactants and other antimicrobial agents. Biosurfactant has the ability to resist corrosion caused by pathogenic microorganisms on metals such as steel. In this study, Biosurfactant producing Lactobacillus lactis were isolatedmand the isolated biosurfactant was coated on to the metal surface. The SEM results revealed that the biosurfactant coated shows less corrosion when compared to the control. The FTIR analysis of the corrosion product of the metal with and without surfactant indicates higher surface activity. The R_{corr} value for the metal coupon with surfactant was measured to be 0.0389 mmpy, whereas R_{corr} value of the metal coupon without surfactant 0.3106 mmpy. Biosurfactant isolated from Lactobacillus lactis reduced the corrosion formed by Thiobacillus sp.

KEYWORDS: Biosurfactant, corrosion, Lactobacillus, Thi0bacillus



I. INTRODUCTION

Corrosion of materials is an electrochemical process which involves oxidation and reduction (Kumar, 2008). Deterioration of metals from corrosion is protected by inhibitors such as organic, inorganic and mixed inhibitors (Raval and Mannari, 1994). Physical and chemical parameters affect the inhibitors efficiency. (Abbasov et al., 2013). Corrosion of iron and steel cause billions of dollars each year. (Noor El-Din, 2009). Inhibitors are used in low concentrations because for its cost affectivity. Selecting effective and flexible inhibitors is a difficult task. Material losses are caused due to corrosion (Wei et al., 2003).

The deteriorating of metal in a specific environment is the rate of corrosion. Corrosion rates are calculated by Mils per year (mpy). The number of millimeters it penetrates. To convert corrosion rate to mils per year and to metric equivalent millimeter per year (mm/y), you can use the following equation:

ISSN- 2394-5125 VOL 7, ISSUE 13, 2020

1 mpy (Mils per year) = 0.0254 mm/y = 25.4 microm/ y

To calculate the corrosion rate from metal loss (Sundjono et al., 2018):

mm /y = 87.6 x (W / DAT)

Where:

W = weight loss in milligrams
D = metal density in g /cm^3
A = area of sample in cm^2
T = time of exposure of the metal sample in hours

Some of corrosion-causing microorganism are Desulfovibrio salixigens, D. vulgaris and D. desulfuricans. Sulfate-reducers require lower potential for growth and this can achieved by production of small amount of hydrogen sulfide (Kobrin, 1993). Corrosion inhibition is a complex process and depends on adsorption onto surface by the surfactant molecule. Surfactants strongly adsorb onto surfaces and are anti-corrosion on surfaces. (Stein and Gellman, 1992; Free, 2002). The advantages of surfactant inhibitors are that they easily adsorb onto surfaces and has high inhibition efficiency and low toxicity (Azhar et al., 2001).

Biosurfactant producing lactobacilli are used because of its probiotic effect and also they are generally recognized as safe microorganism (GRAS) (Sambanthamoorthy et al., 2014). They also have antimicrobial and antiadhesive properties (Duarte et. al., 2014).

To evaluate anti-corrosion performance of protective coating, Electrochemical Impedance Spectroscopy (EIS) is the best method with short testing time. It is measured by applying an AC potential to a electrochemical cell and measuring the current that passes through it (Mansfeld, 1990). Fourier transform Infrared (FTIR) analysis is performed to characterize the surface of metal plates and identify the functional groups present. The formation of corrosion can be identified by the presence of iron oxide that is rust and other metal carbonates or chlorides in the region 800 – 1100 cm^{-1} (Neufeld and Cole, 1997).

In the present study, biosurfactant producing lactobacillus was isolated and their anti-corrosion ability were performed on Thiobacillus sp. The isolated surfactant were characterized for various physical and chemical parameters. The corrosion inhibition effect for the surfactant were analyzed on Thiobacillus sp.

II. MATERIALS & METHODS

Isolation of Lactobacillus sp.

Lactobacillus was cultured on MRS agar which was isolated from homemade curd, incubated at 28 ˚C overnight. Pure colonies were sub-cultured on fresh MRS agar plates.

Isolation of Iron Oxidizing Bacteria

Rust sample was collected and was inoculated in Lyngby's broth and incubated at 37 ℃ for 48 hours. After 48 hours 1 ml of Lyngby's broth was added to 20 ml of Iron oxidizing bacteria isolation broth, and incubated at 37 ℃ for 24 hours. After which the cultures were streaked on iron oxidizing bacteria agar media (Atlas, 2010).

Biosurfactant Production

For the production of bio-surfactants, the isolated lactobacillus culture was incubated in large quantity in MRS broth at 28 ℃ for 24 hours. Then the solution was extracted and purified as per standard procedure and purified (Adamu et al., 2015).

ISSN- 2394-5125 VOL 7, ISSUE 13, 2020

Screening of Biosurfactant

Oil spread technique

For the identification of bio surfactant by oil spread technique, distilled water and oil was added individually onto a petri-plate. Then a tiny amount of the supernatant was added to it. The diameter of a clear zone was measured. (Anayata Sharma et al., 2014).

Emulsification index test

A mixture of the supernatant sample and oil (petrol) was taken in test tube and vortexed and incubated at RT for 24 hours. The emulsification index (E_{24}) was calculated (Anayata Sharma et al., 2014).

$$E_{24} = \frac{\text{Total height of the emulsion}}{\text{Total height of the aqueouslayer}} X = 100$$

Drop collapse test

The flattening and spreading of the bio surfactant and water on a film was observed and the difference were recorded. (Tahmourespour et al., 2011).

Biochemical characterization

The isolated colonies were identified based on morphology and various standard biochemical characteristics (Holt et al., 1994).

DNA isolation for molecular identification

DNA was isolated as per standard procedure and stored in TE buffer (Goodfellow et al., 1991)

Sequencing

PCR reaction was performed as prescribed by the manufactures protocol (QIAGEN) in Bio-Rad iQ 5 RTPCR. Bacteria were identified by comparing the sequences using NCBI BLAST program (Embley, 1991).

Enumeration of H_2S Producing organisms by MPN Test

Most Probable Number (MPN) was performed estimate the concentration of viable microorganisms in a sample using Lyngby's broth. It was performed for sample as per standard procedure. (Manja et al.,2001).

Corrosion Inhibition using Surfactant

For this study the isolated colony from the rusted iron plate is inoculated into the 500 ml Lyngby's broth and incubated at 37 ℃ for 48 hours. Metal coupons of uniform size were immersed on to sterile media inoculated with Thiobacillus after taking their dry weight. The broth was incubated for 30 days at 37. Another coupon was taken and dried weight was checked and the coupon was coated with Surfactant mixed with thinner enamel and dried for 24 hours, and placed in Lyngby's broth and kept for incubation for 30 days at 37 ℃. After 30 days the coupons were taken, dried and the weight was checked (Rafiquee et al., 2008).

Corrosion Studies

Weight Loss Method

Weight loss measurements were used for studying the corrosion of mild steel in Lyngby's broth inoculated with Iron Oxidizing bacteria. The samples were completely suspended in the broth and the weight loss measurement was recorded every three days using digital weighing balance and exposure for 15 days. (Chen et al., 2005).

ISSN- 2394-5125 VOL 7, ISSUE 13, 2020

The dried weight of the coupons is calculated by the formula:

Initial weight of the metal ~~(X)~~ ▶

Final weight of the metal ~~(Y)~~ ▶

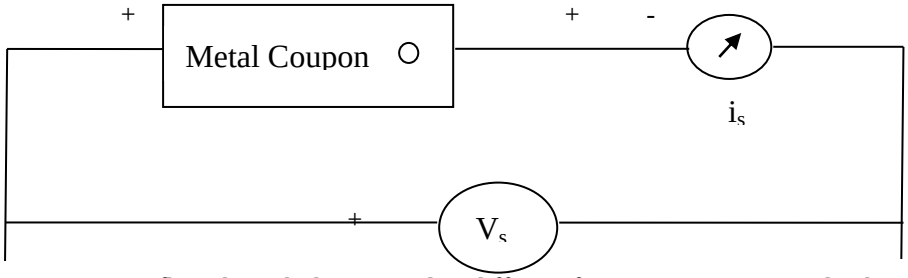
So weight loss for 30 days ~~X Y~~ ▶(A)

Therefore the weight loss determination for 1 year $\frac{A \times 365}{30}$ ▶(Z)

$$R_{corr}(mmpy) = 87.6\ X\ \frac{\text{Change in weight}}{\text{density}}\ X\ \text{Area}\ X\ \text{Time}$$

Impedance Test

Electrochemical Impedance Spectroscopy (EIS) measurements were carried out in duplicate using a frequency range of 100 kHz to 0.001 Hz with an AC wave of ±10 mV after 30 minutes of stabilization in the open-circuit potential. The experiments were performed in the same electrolytic media earlier used for the E_{ocp} measurements and polarization tests. (Zhang et al., 1993). The EIS data were also analyzed to verify the influence of Surfactant on the corrosion of carbon steel, and the results are shown in plots for 12 days of exposure. Impedance (Z) of a material can be obtained from the following circuit.



Here, current flow through the material at different frequencies is measured. Then the impedance is calculated using the formula

$$\mathbf{Z} = \frac{\mathbf{Vs}}{\mathbf{i}}$$

Scanning Electron Microscope (SEM) analysis

The metal coupons coated with surfactant and without surfactant were subjected to SEM analysis to check the material corrosion on the surface of metal coupons. The SEM analysis was carried out in TESCAN VEGA model.

Fourier transform infrared spectroscopy (FTIR) analysis

The samples were analyzed by FTIR spectrum for identification of functional groups in the samples. The FTIR spectra was recorded at transmission mode scan. (Clark and Hester, 1996).

III. RESULTS & DISCUSSION

Isolation and characterization of Lactobacillus sp.

Lactobacillus was isolated from homemade curd sample on MRS agar media. The culture was subjected to differential staining procedure and Gram's staining was done. The isolate was gram positive, rod shaped, smooth, round, translucent, creamy off white colonies. The strain obtained was identified to generic level based on biochemical characteristics (Table 1 and 2). The culture were sequenced and the sequences were deposited in

ISSN- 2394-5125 VOL 7, ISSUE 13, 2020

GenBank. The organism was identified as Lactobacillus lactis (Table 3). Beasley et al., 2004 also reported the same.

Characterization of biosurfactants

The biosurfactant produced (Figure 1) were characterized for its stability. Oil spreading technique showed the displacement of the oil, and the diameter of the clear zone formed was measured as 2 cm. The oil displacement test is an indirect measurement of surface activity of bio surfactants: a larger clear area is correlated with higher surface activity. The emulsification index (E_{24}) obtained was 62.5%. (Figure 2). The emulsification index value is more than 50% whis is also suggested by Willumsen et al., 1996. The emulsification index of the biosurfactant produced by Lactobacillus sp. is 62.5%. The biosurfactant indicated its effect on reduction in surface tension from droplet size being 1 mm more than of water.

The characterization of biosurfactant through FTIR shows that the biosurfactant shas higher surface activity due to the presence of C-C and C=C groups in the region 1800-1200 cm^{-1}. Therefore could easily attach to metal surfaces. Thus a good bacterium with antimicrobial product is effective against a corrosion bacteria.

Isolation and characterization of Iron Oxidizing Bacteria

The test organism for the corrosion studies- IOB was isolated from rust using Lynby's broth and incubated at 37 ºC for 48 hours. After 48 hours the appearance of black color confirms H_2S production in the media by the isolate (Figure 3). The Iron oxidizing bacteria was isolated and confirmed using Iron oxidizing bacteria isolation media. After 24 hours of incubation, the tubes showed turbidity, indicating the presence of Iron oxidizing bacteria (Figure 4). The isolated iron oxidizing bacteria was identified based on its morphological and biochemical characteristics (Table 4). After gram staining, the smear was observed under microscope at 100 X magnification and pink colored rod shaped bacteria were observed indicating gram negative bacteria. The morphological characteristics were observed. Biochemical characteristics such as Catalase, Oxidase showed positive test, Carbohydrate utilization test for glucose shoed acid production, for lactose and sucrose showed no acid production, Nitrate reduction test, H_2S production showed positive test (Table 5). Molecular identification of the isolate was done by 16Sr RNA sequencing and the sequences were subjected to BLAST analysis. Based on the sequence homology the organism was identified as Thiobacillus sp. The sequence was submitted to GenBank (Table 6). The total number of organisms in the sample was enumerated by using MPN test. By combining the positive tubes for H_2S production, the number of organisms was found to be 11 per 100 ml of the media, with a maximum MPN index of 29 and a minimum index of 4 for broth without surfactant. Similarly, the number of organisms was found to be 2 per 100 ml of the media, with a maximum MPN index of 4 and a minimum index of 0 for broth with surfactant.

Due to the acidophilic and neutrophilic spices there is production of sulfuric acis on concrete surfaces (Okabe et al., 2006). In this study also Thiobacillus sp., an IOB and SOB was isolated from iron oxide deposits of the rust samples.

Scanning Electron Microscopy (SEM)

Based on the results of SEM analysis, under microbial action corrosion is aggravated which is the root cause of the observed corrosion damage (Figure 5). On the control mild steel (without surfactant) there was less protective layer and more corrosion. On the surfactant coated sample it had thicker layer and less corrosion, which shows that it is protected from corrosion. This can be seen in weight loss experiment as well. The SEM image depicts the corrosive action of the isolates. Presence of pits, hairline cracks and profound destruction of the metal surface were observed. Destruction of the metal surface is because of the acid production and the deposition of the iron oxides on the surface. Tribollet et al., (2013) stated that the corrosion is trans-granular since he observed corrosion on boundaries at initial stages. When the passive layer breaks down he observed holes to form. So the surfactants protect the surface from corrosion and also slow down the process. Organic acids produces by Thiobacillus sp., is not strong enough to dissolve this metal surfaces.

ISSN- 2394-5125 VOL 7, ISSUE 13, 2020

Corrosion Studies

The weight loss study was carried out using steel coupons in Lynby's broth (Figure 6). The Corrosion rate was found by measuring the weight loss in grams and converted to mmpy. The R_{corr} value for the metal coupon with surfactant was measured to be 0.0389 mmpy, whereas R_{corr} value of the metal coupon without surfactant 0.3106 mmpy (Table 7). The corrosion rate was found to be lesser in the system with surfactant. Thus it is proved that the surfactant of Lactobacillus lactis inhibit the formation of Biofilm by Thiobacillus sp.

The bode plots for sterile and experimental system for metal is shown in figure. It can be seen that the Rct value for the control system is 2.3 K ohm/cm2. In the presence of bacteria, the resistance is in the range between 6.6 and 7.9 Ohm/cm2. The EIS data (Table 8 & 9) were analyzed for 12 days (Figure 7) and shows that the in the presence of surfactant the polarization resistance of carbon increased independent of the exposure time. The variation in the impedence is due to the corrosive properties of Thiobacillus sp., on metal surface. The presence of surfactant on the metal surface clearly shows similarity with the control system. It is evident that the inhibition of the corroding bacteria in adhering on to the metal surface.

The FTIR pattern of the corrosion product of the metal with and without surfactant reveals the bands at 1642 cm-1 is due to C=C, 1408 cm^{-1} is CH_2 and 1546 cm^{-1} is for C=O (Figure 8). These indicated the presence of surfactant coated onto the surface and these bands were missing on non coated surface. There are shifts in the frequency due to stain in the carbonyl ring (Silverstein et al., 1978). Here in this study the band on 1642 cm^{-1} indicates the presence of aromatic nuclei in the presence of surfactant. Whereas the band 1708 cm^{-1} confirms the presence of carboxylic acid residues without the presence of surfactant. It is evident that the acid production is dominated by the Thiobacillus sp., in the absence of Lactobacillus.

At low concentration inhibition was achieved for piperidin-4-one but higher was required for cyclohexanone (Sankarapapavinasam et al., 1991). Currently the supernatant surfactant isolated from Lactobacillus sp., (Raw and crude extract) showed much inhibition in the formation of Thiobacillus layer on the metal surface. This result confirms that the surfactant did not permit Thiobacillus sp., to form a film over the metal surface which is the cause of change in the electrochemical properties of the systems (Nguyen et al., 2008). With more exposure time the corrosion product becomes compact (Duan et al., 2008). The variation in the impedence is due to the corrosive properties of Thiobacillus sp., on metal surface. The presence of surfactant on the metal surface clearly shows similarity with the control system. It is evident that the inhibition of the corroding bacteria in adhering on to the metal surface. The surface characterization and localized damage can be studied from impedance. The corrosion resistance increased by coating of surfactant on to the metal surface. The surface resistance of stainless steel without surfactant is comparatively low this is evident by the impedence values. The metal containing surfactant coating showed the best corrosion protection performance compared to the other systems. In weight loss method, for metal coated with surfactant showed 0.0389 grams and without surfactant showed a weight of 0.3106 grams. This implies that the metal coated with surfactant did not allow the corrosion causing bacteria to cause corrosion on to the metal surface, resulting that the surfactant has the ability to inhibit the action of corrosion. The impedance test done for the metal coupons showed that the metal coupon coated with surfactant showed less corrosion when compared with that of the metal without surfactant. With these tests, it was confirmed that the biosurfactant has the ability to inhibit the corrosion on metal surfaces.

Malik et al., (2011), summarized that due the high affinity of surfactant molecules to adsorb onto interfaces to form micelles they are best eco-friendly anti-corrosion substances to protect the materials from corrosion. They can be used in industry to protect metals from corrosion and prevent economic loss. Thus, it can be proved that the surfactants derived from Lactobacillus lactis in the current study is a potent inhibitor of corrosion causing bacteria (Thiobacillus sp.). Thus, metal surfaces can resist corrosion in the presence of surfactant derived from Lactobacillus lactis.

IV. CONCLUSION

In the present study, Biosurfactant produced by Lactobacillus lactis was isolated from homemade curd samples. This probiotic organism has the ability to produce surfactant which is a lipopeptide. This biosurfactant is proved to have inhibitory activity on other microbial flora. The surfactant extracted was confirmed and applied over metal surfaces using thinner, this perhaps inhibit the binding of other microorganisms when the metal surface is exposed to environment. Thus it is proved that the surfactant extracted acts as an effective biocide of corrosion. This is demonstrated with exposure of metal surface to iron oxidizing bacteria such as Thiobacillus by various

tests such as drop collapse test, emulsification index and oil displacement method. Thiobacillus It is evident that the surfactant acts as a protective layer and inhibits the adherence of corroding bacteria on to the metal surface.

V. REFERENCES

[1] Abbasov, V.M., El-Lateef, H.M.A., Aliyeva, L.I., Qasimov, E.E., Ismayilov, I.T. and Khalaf, M.M., 2013. A study of the corrosion inhibition of mild steel C1018 in CO2-saturated brine using some novel surfactants based on corn oil. Egyptian Journal of Petroleum, 22(4), pp.451-470.

[2] Adamu, A., Ijah, U.J., Riskuwa, M.L., Ismail, H.Y. and Ibrahim, U.B., 2015. Study on biosurfactant production by two Bacillus species. International Journal of Scientific Research in Knowledge, 3(1), p.13.

[3] Anayata Sharma, Jitesh Soni,Gurveer Kaur,Jaspreet Kaur. 2014. A study on biosurfactant production in Lactobacillus and Bacillus sp. International Journal of current Microbiology and Applied Sciences, 3(11).723-733

[4] Atlas R.M., 2010. Handbook of microbiological media. CRC press.

[5] Beasley, S., 2004. Isolation, identification and exploitation of lactic acid bacteria from human and animal microbiota.

[6] Chen, X.H., Chen, C.S., Xiao, H.N., Cheng, F.Q., Zhang, G. and Yi, G.J., 2005. Corrosion behavior of carbon nanotubes–Ni composite coating. Surface and Coatings Technology, 191(2-3), pp.351-356.

[7] Clark, R.J.H. and Hester, R.E. eds., 1996. Biomedical applications of spectroscopy (Vol. 25). Chichester, UK: Wiley.

[8] Duan, J., Wu, S., Zhang, X., Huang, G., Du, M. and Hou, B., 2008. Corrosion of carbon steel influenced by anaerobic biofilm in natural seawater. Electrochimica Acta, 54(1), pp.22-28.

[9] Duarte, C., Gudiña, E.J., Lima, C.F. and Rodrigues, L.R., 2014. Effects of biosurfactants on the viability and proliferation of human breast cancer cells. AMB express, 4(1), p.40.

[10] El Azhar, M., Mernari, B., Traisnel, M., Bentiss, F. and Lagrenée, M., 2001. Corrosion inhibition of mild steel by the new class of inhibitors [2, 5-bis (n-pyridyl)-1, 3, 4-thiadiazoles] in acidic media. Corrosion Science, 43(12), pp.2229-2238.

[11] Embley, T.M., 1991. The linear PCR reaction: a simple and robust method for sequencing amplified rRNA genes. Letters in Applied Microbiology, 13(3), pp.171-174.

[12] Free, M.L., 2002. Understanding the effect of surfactant aggregation on corrosion inhibition of mild steel in acidic medium. Corrosion Science, 44(12), pp.2865-2870.

[13] Goodfellow, M., Chun, J., Stackebrandt, E. and Kroppenstedt, R.M., 2002. Transfer of Tsukamurella wratislaviensis Goodfellow et al., 1995 to the genus Rhodococcus as Rhodococcus wratislaviensis comb. nov.. International journal of systematic and evolutionary microbiology, 52(3), pp.749-755.

[14] Holt, J.G., Krieg, N.R. and Sneath, P.H., 1994. Bergey's manual of determinative bacteriology.

[15] Kobrin, G., 1993. A practical manual on microbiologically influenced corrosion. G. Kobrin, Editor, published 1993 by NACE, 250.

[16] Kumar, A., 2008. Corrosion inhibition of mild steel in hydrochloric acid by sodium lauryl sulfate (SLS). Journal of chemistry, 5(2), pp.275-280.

[17] Malik, M.A., Hashim, M.A., Nabi, F., Al-Thabaiti, S.A. and Khan, Z., 2011. Anti-corrosion ability of surfactants: a review. Int. J. Electrochem. Sci, 6(6), pp.1927-1948.

[18] Manja, K.S., Sambasiva, R., Chandrashekhara, K.V., Nath, K.J., Dutta, S., Gopal, K., Iyengar, L., Dhindsa, S.S. and Parija, S.C., 2001. Report of study on H2S test for drinking water. UNICEF, New Delhi.

[19] Mansfeld, F., 1990. Electrochemical impedance spectroscopy (EIS) as a new tool for investigating methods of corrosion protection. Electrochimica Acta, 35(10), pp.1533-1544.

[20] Neufeld, A.K. and Cole, I.S., 1997. Using Fourier transform infrared analysis to detect corrosion products on the surface of metals exposed to atmospheric conditions. Corrosion, 53(10), pp.788-799.

[21] Nguyen, T.M.P., Sheng, X., Ting, Y.P. and Pehkonen, S.O., 2008. Biocorrosion of AISI 304 stainless steel by Desulfovibrio desulfuricans in seawater. Industrial & engineering chemistry research, 47(14), pp.4703-4711.

[22] Noor El-Din, M.R., 2009. Corrosion inhibition efficiency of some new surfactants on carbon steel type H-11 in 1 M hydrochloric acid solution. Journal of dispersion science and technology, 30(7), pp.1027-1035.

[23] Okabe, S., Odagiri, M., Ito, T. and Satoh, H., 2007. Succession of sulfur-oxidizing bacteria in the microbial community on corroding concrete in sewer systems. Appl. Environ. Microbiol., 73(3), pp.971-980.

[24] Rafiquee, M.Z.A., Saxena, N., Khan, S. and Quraishi, M.A., 2008. Influence of surfactants on the corrosion inhibition behaviour of 2-aminophenyl-5-mercapto-1-oxa-3, 4-diazole (AMOD) on mild steel. Materials Chemistry and Physics, 107(2-3), pp.528-533.

[25] Raval, D.A. and Mannari, V.M., 1994. IMIDAZOLINE DERIVATIVES AS CORROSION-INHIBITORS. RESEARCH AND INDUSTRY, 39(2), pp.94-95.

[26] Sambanthamoorthy, K., Feng, X., Patel, R., Patel, S. and Paranavitana, C., 2014. Antimicrobial and antibiofilm potential of biosurfactants isolated from lactobacilli against multi-drug-resistant pathogens. BMC microbiology, 14(1), p.197.

[27] Sankarapapavinasam, S., Pushpanaden, F. and Ahmed, M.F., 1991. Piperidine, piperidones and tetrahydrothiopyrones as inhibitors for the corrosion of copper in H2SO4. Corrosion Science, 32(2), pp.193-203.

[28] Silverstein, R.M. and Bassler, G.C., 1962. Spectrometric identification of organic compounds. Journal of Chemical Education, 39(11), p.546.

[29] Sundjono, S., Priyotomo, G., Nuraini, L. and Prifiharni, S., 2018. Corrosion Behavior of Mild Steel in Seawater from Northern Coast of Java and Southern Coast of Bali, Indonesia. Journal of Engineering and Technological Sciences, 49(6), pp.770-784.

[30] Stein, T.M. and Gellman, S.H., 1992. Synthesis and aggregation properties of a new family of amphiphiles with an unusual headgroup topology. Journal of the American Chemical Society, 114(10), pp.3943-3950.

[31] Tahmourespour, A., Salehi, R. and Kermanshahi, R.K., 2011. Lactobacillus acidophilus-derived biosurfactant effect on gtfB and gtfC expression level in Streptococcus mutans biofilm cells. Brazilian Journal of Microbiology, 42(1), pp.330-339.

[32] Tribollet, B., Kittel, J., Meroufel, A., Ropital, F., Grosjean, F. and Sutter, E.M.M., 2014. Corrosion mechanisms in aqueous solutions containing dissolved H2S. Part 2: Model of the cathodic reactions on a 316L stainless steel rotating disc electrode. Electrochimica Acta, 124, pp.46-51.

[33] Tuvell, M.E., Kuehnhanss, G.O., Heidebrecht, G.D., Hu, P.C. and Zielinski, A.D., 1978. AOS—An anionic surfactant system: Its manufacture, composition, properties, and potential application. Journal of the American Oil Chemists' Society, 55(1), pp.70-80.

[34] Wei, Z., Duby, P. and Somasundaran, P., 2003. Pitting inhibition of stainless steel by surfactants: an electrochemical and surface chemical approach. Journal of colloid and interface science, 259(1), pp.97-102.

[35] Willumsen, P.A. and Karlson, U., 1996. Screening of bacteria, isolated from PAH-contaminated soils, for production of biosurfactants and bioemulsifiers. Biodegradation, 7(5), pp.415-423.

[36] Zhang, M.I.N., Willison, J.H.M., Cox, M.A. and Hall, S.A., 1993. Measurement of heat injury in plant tissue by using electrical impedance analysis. Canadian journal of botany, 71(12), pp.1605-1611.

Tables & Figures

Table 1. Morphological characteristics of Lactobacillus lactis

S. No	Morphological characteristics	Observations
1.	Surface	Smooth, creamy
2.	Opacity	Translucent
3.	Colour	Off white
4.	Motility	Motile
5.	Gram staining	Positive
6.	Cell shape	Rods

Table 2. Biochemical characteristics of Lactobacillus lactis

S. No	Biochemical Tests performed	Observations
1.	Indole	Negative
2.	Methyl red	Positive
3.	Vogues Proskauer	Negative
4.	Citrate	Negative
5.	Catalase	Negative
6.	Oxidase	Negative
7.	Starch hydrolysis	Positive

ISSN- 2394-5125 VOL 7, ISSUE 13, 2020

8.	Casein hydrolysis	Positive

Table 3. Molecular Identification of the Lactobacillus lactis

S.No.	BLAST results	Gen Bank accession number
1.	**Lactobacillus lactis**	BankIt2080338

Table 4. Biochemical Characteristics of the Thiobacillus sp.

S. No	Biochemical Tests	Results
1.	Gram Staining	Gram negative rods
2.	Motility test	Motile with polar flagella
3.	Oxidase test	Positive
4.	Catalase test	Positive
5.	Carbohydrate utilization test: Glucose Lactose Sucrose	 Acid production (+) No acid production(-) No acid production(-)
6.	Nitrate Reduction test	Positive
7.	H_2S production	Positive

Table 5. Molecular Identification of the Thiobacillus sp.

S. no.	BLAST results	Gen Bank accession number
1.	**Thiobacillus sp.**	EF363525

Table 6. Enumeration by MPN test (H_2S Production)

Combination of positive	MPN index/100 ml	95% confidence level
3-1-0	11	Above-4 Below-29
2-0-0	2	Above-4 Below-0

Table 7. Weight loss study of the metal

S. No.	Metal Coupons	Weight Loss		Weight Loss For 15 Days(g)	Weight Loss For 1year(g)	R_{corr} (mmpy)
		Initial Weight(g)	Final Weight(g)			
1.	Without Surfactant	16.985	16.602	0.383	4.659	0.3106
2.	With surfactant	18.182	18.134	0.048	0.584	0.0389

ISSN- 2394-5125 VOL 7, ISSUE 13, 2020

Table 8. Electrochemical Impedance Measurement

Frequency		V_s	Control		Without Surfactant		With Surfactant	
			I	$Z=V/i$	I	$Z=V/i$	I	$Z=V/i$
0	0	1v	1.0	1000	0.3	3333	1	1000
10^0	1	1v	1.1	909	0.4	2500	1.4	714
10^1	10	1v	5.19	192	5	200	5.3	188
10^2	100	1v	5.93	168	5.9	169	5.9	169
10^3	1000	1v	4.83	207	4.8	208	4.75	210
10^4	10000	1v	3.81	262	3.82	261	3.9	256
10^5	100000	1v	1.35	740	1.1	909	1.3	769

Table 9. Comparison of Impedance and Frequency

Frequency (V)	Control Impedance (Z1) (Ohm)	Metal Without Surfactant Impedance (Z2) (Ohm)	Metal Coated With Surfactant Impedance(Z3) (Ohm)
0	1000	3333	1000
1	909	2500	714
10	192	200	188
100	168	169	169
1000	207	208	210
10000	262	261	256
100000	740	909	769

Fig. 1. Biosurfactant production

Fig. 2. Test tube showing emulsification index of biosurfactant

Fig. 3. Isolation of Thiobacillus sp., in Lynby's Broth

ISSN- 2394-5125 VOL 7, ISSUE 13, 2020

Fig. 4. Growth of Thiobacillus sp., in Iron Oxidising Bacteria Media

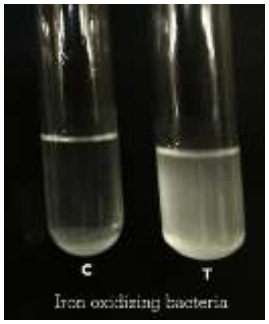


Fig. 5. SEM images of metal coupons a) coated with biosurfactant, b) without biosurfactant

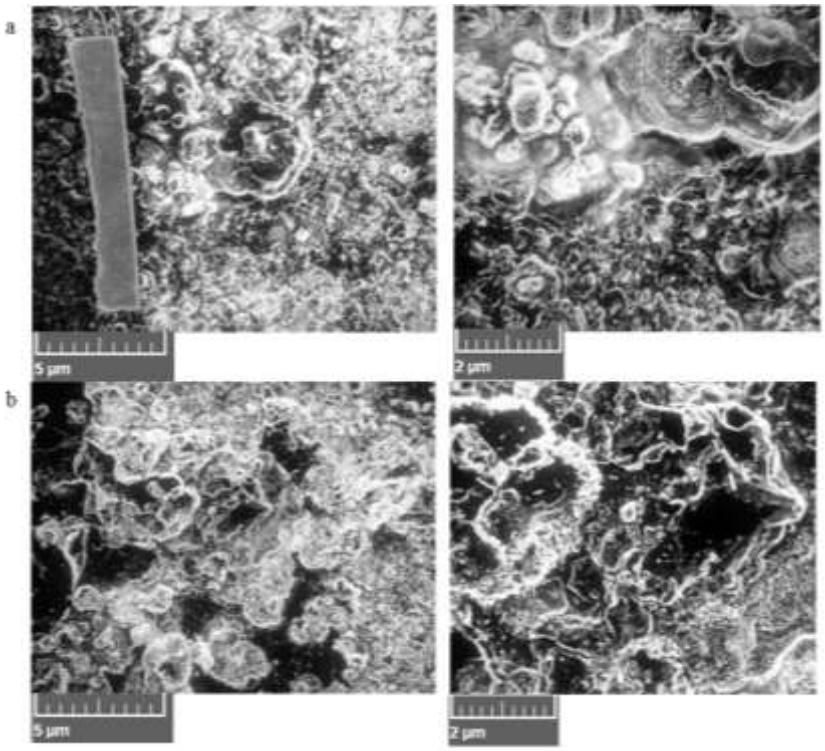


Fig. 6. Corrosion study -Weight Loss method

ISSN- 2394-5125 VOL 7, ISSUE 13, 2020

Fig. 7. EIS plot

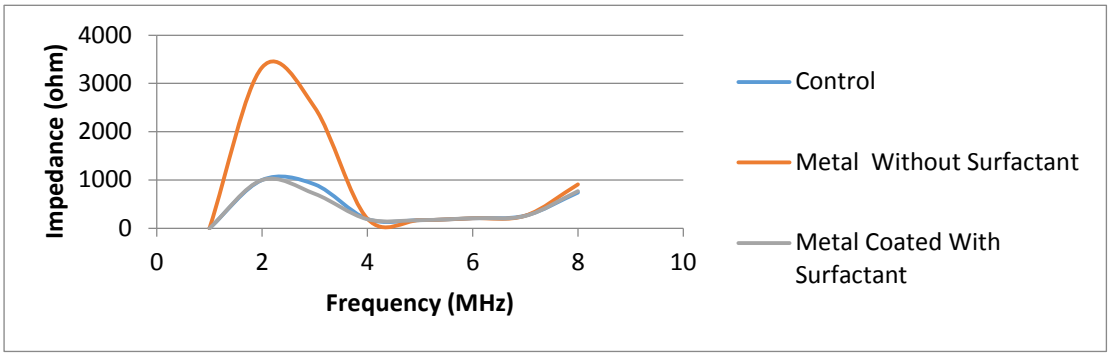
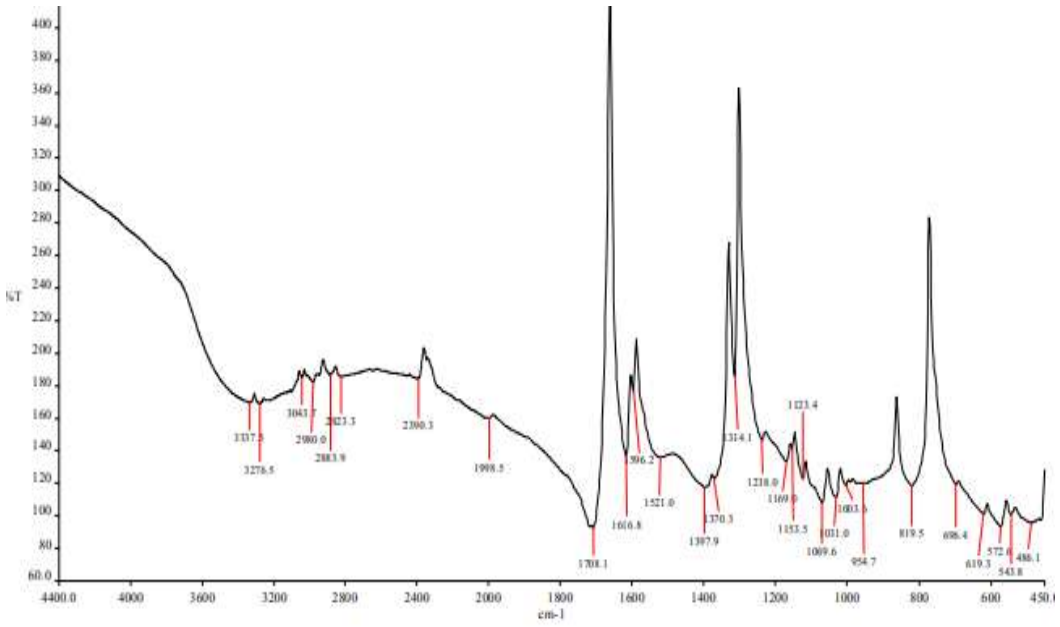


Fig. 8. FTIR pattern of Corrosion product a) without surfactant, b) with surfactant

a



ISSN- 2394-5125 VOL 7, ISSUE 13, 2020