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Research Article
Effect of Lemongrass (*Cymbopogon citratus*) Essential Oil on Biofilm-biocorrossion in Formation Water Environment

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Abstract

Background and Objective: Microbial accumulation in oil industry's pipeline system would eventually leads to biofilm formation, which could initiate biocorrosion. Biofilm-biocorrosion caused great loss in industry and is essential to be included in mitigation program. Essential oils extracted from plants are known to have anti-microbial properties. This study aimed to further investigate the effect of lemongrass (*Cymbopogon citratus*) essential oil in emulsion and microemulsion form against biofilm in formation water environment. **Materials and Methods:** Thermodynamic stability of microemulsion was observed with centrifugation, heating-cooling cycle and freeze-thaw cycle method and confirmed with particle size measurement. **Results:** Lemongrass essential oil treatment was able to prevent both biofilm and planktonic bacterial growth and eradicate biofilm. Minimum inhibitory concentration (MIC) of emulsion was observed at essential oil concentration of 0.06% (v/v). The minimum biofilm inhibitory concentration of emulsion and microemulsion assayed on carbon steel were 0.06 and 0.03%, respectively. Minimum biofilm eradicating concentration of emulsion and microemulsion were both 1% with eradication percentage of 53.03 and 88.39%, respectively. Biocorrosion mitigation potential of lemongrass essential oil was most effective (96.786% inhibition) at 2% (v/v) after 6 h of exposure. **Conclusion:** Essential oil in emulsion and microemulsion form could perform as alternative to chemical biocide in mitigating biofilm-biocorrosion.

Key words: Biofilm, biocorrosion, MIC, lemongrass, carbon steel, mitigation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Indonesia's energy needs are increasing along with economic and population growth. Meanwhile, oil production is decreasing from 287.1 million barrels in 2006 to 251.87 million barrels in 2015[1]. Microbial Enhanced Oil Recovery (MEOR) is one of tertiary methods that increases oil production by harnessing microbial activity in petroleum reservoirs[2]. MEOR can increase petroleum production, but it also leads to microbial accumulation in oil industry pipeline. Oil production process and the reservoir itself can also contribute to the microbial accumulation. Microbial cells tend to form biofilm which has caused many problems such as pipe clogging, biofouling, oil souring and microbiologically influenced corrosion (MIC)[3]. The MIC is estimated to cause 40% of internal pipeline corrosion in petroleum industry[4] and resulted in financial loss of up to 10 billion dollars/year[5].

Biofilm-biocorrosion mitigation in oil industrial pipeline systems has been attempted using biocides e.g., aldehydes, chlorines and amines. However, biocides are toxic, corrosive, expensive and inherently difficult to degrade. Biocides use has also been shown to cause microbial resistance in indigenous bacteria[6]. These negative environmental impacts have been a great problem in many industries, particularly petroleum industry. Thus, it is important to find a safe, inexpensive and environmentally friendly alternative to chemical biocides.

Essential oil extracted from plants can exhibit antibacterial or antifungal activities, making it a potential biocide[7]. Lemongrass (*Cymbopogon citratus*) contains a high concentration of essential oil in the range of 0.4-0.6%[8]. Lemongrass essential oil (LEO) has been investigated for its ability to inhibit the growth of biofilm and planktonic bacteria, eradicate mature biofilm and prevent corrosion on metals especially in medical and food industry[9]. These abilities have been studied for sulfate reducing bacteria[9] but the potential was yet to be fully explored against indigenous microbial consortium in formation water.

This study aimed to determine the effective concentration and exposure period of lemongrass essential oil to mitigate biofilm and biocorrosion growing in formation water environment. The optimum concentration and exposure period was expected to give a detrimental effect to biofilm of formation water indigenous bacteria from South Sumatran oil well and reduce corrosion damage on ST-37 carbon steel surface.

MATERIALS AND METHODS

Essential oil preparation: Lemongrass essential oil was obtained from cv. Pavettia Atsiri, Subang and West Java. Essential oil was prepared in emulsion and microemulsion form according to Adukwu *et al.*[10]. Thermodynamic stability was tested using centrifugation, heating cooling cycle and freeze thaw cycle method[11]. Microemulsion particle size was measured using particle size analyzer.

Bacterial isolates: Microbial isolates used in this study were obtained from formation water taken from South Sumatran oil well.

Minimum inhibitory concentration (MIC) assay: Formation water was inoculated with 10% bacterial isolates and incubated for 6 days at 70°C. A 20 µL aliquot of activated culture and 180 µL of essential oil in varying concentration were added to each well of microtiter plate and incubated at 70°C. Formation water and tween 80 was mixed as negative control. Bacterial growth was measured with BIO-RAD ELISA Reader (OD595) before and after 48 h of incubation period. MIC was determined as minimum concentration where Δ absorbance of the treatment was less or equal to that of negative control[7].

Minimum biofilm inhibitory concentration (MBIC) assay: Formation water was inoculated with 10% bacterial culture, supplemented with 1% glucose and incubated for 6 days at 70°C. A 100 µL aliquot of the activated culture and 100 µL of essential oil in varying concentration was added to each well of microtiter plate and incubated for 48 h at 70°C. Subsequently, liquid culture was carefully removed without disrupting the formed biofilm on plastic surface. Microtiter plate was washed two times with PBS buffer and heated at 70°C for 15 min. Each well was added with 0.1% of crystal violet, incubated for 30 min and washed three times with PBS buffer. Two hundred microliter of 70% ethanol was added to each well to dissolve remaining crystal violet. Absorbance of dissolved crystal violet was measured at 595 nm using BIO-RAD Elisa Reader. The MBIC was determined as minimum concentration where Δ absorbance of treatment was less or equal to that of negative control[7].

Minimum biofilm eradicating concentration (MBEC) assay: Biofilm on microtiter plate was prepared as previously described without adding essential oil. After 48 h of

incubation, liquid culture was removed and microtiter plate was washed with PBS buffer. Then, 200 µL of essential oil emulsion or microemulsion was added to each well and microtiter plate was incubated for 6 h at 70°C. Crystal violet staining and absorbance measurement were done as previously described. The MBEC was determined as minimum concentration where Δ absorbance of treatment was at least 50% less than negative control, indicating 50% eradication of biofilm[7].

ST-37 carbon steel coupons preparation: The ST-37 carbon steel (10×10×1.8 mm^3) was scoured with 200, 400, 600 and 800 grade sandpapers[12]. Then, it was decreased with 98% ethanol and soaked in acetone. The coupons were dried for 2 h in 70°C oven and autoclaved at 121°C for 15 min.

Biofilm-biocorrosion inhibition by essential oil: Biofilm was grown on ST-37 carbon steel surface by soaking it in a mixture of formation water, 4.6% (v/v) of molasses, 0.47% (w/v) of diammonium phosphate and 0.5% (w/v) of NPK for 14 days at 70°C as previously described[13]. Essential oil was then added in the concentration of 0.5, 1 and 2% (v/v), with varying exposure time of 2, 6 and 10 h.

Biofilm morphological observation: Steel coupons were soaked in 4% formaldehyde in PBS buffer and ethanol with the concentration of 70, 75, 80, 85, 90 and 98%, consecutively[13] Biofilm on carbon steel surface was visualized using scanning electron microscope (SEM).

Biocorrosion morphological observation: Steel plates were cleaned with PBS buffer, 98% ethanol and acetone and dried in 70°C oven for 2 h[13]. Corrosion on steel coupons was observed with scanning electron microscope (SEM).

Corrosion rate measurement: The carbon steel plates were weighed before and after incubation using analytical scale. Corrosion rate was calculated by using Eq. 1[14]:

$$\text{Corrosion rate} = \frac{C \times W}{A \times T \times D} \quad (1)$$

C = Constant
T = Exposure period (h)
A = Area (cm^2)
W = Weight loss (g)
D = Density (g cm^{-2})

Inhibitor efficiency (IE) calculation: Inhibitor efficiency was calculated by using Eq. 2[15]:

$$IE = \frac{W_0 \times W_1}{W_0} \times 100 \quad (2)$$

IE = Inhibitor efficiency
W_o = Steel weight loss without biocide exposure (g)
W_1 = Steel weight loss with biocide exposure (g)

Statistical analysis: Analysis of variance (ANOVA) was used as the statistical method to analyse the data.

RESULTS

Minimum inhibitory concentration (MIC) assay: Minimum inhibitory concentration assay was conducted to assess lemongrass oil emulsion inhibition toward planktonic cells. Planktonic cells of biofilm-forming culture were incubated with varying concentration of oil emulsion. Inhibitory effect of oil emulsion was calculated based on bacterial growth. The assay showed that minimum concentration of 0.06% (v/v) of lemongrass essential oil emulsion was required to inhibit the planktonic cell growth (Fig. 1).

Minimum biofilm inhibitory concentration (MBIC) assay: This assay was conducted to determine the minimum concentration of lemongrass essential oil in inhibiting the formation of biofilm on plastic microtiter plate well surface. Potential biofilm forming culture were incubated with varying concentration of essential oil, either in emulsion or microemulsion form and biofilm was measured after 48 h. By comparing to control, result showed that MBIC of lemongrass essential oil are 0.06% (v/v) for emulsion-form and 0.03% (v/v) for microemulsion-form (Fig. 2). This result showed that lower concentration of essential oil in microemulsion are needed to inhibit the formation of biofilm.

Minimum biofilm eradicating concentration (MBEC) assay: This assay was conducted to determine the minimum concentration of lemongrass essential oil in eradicating pre-developed biofilm. After establishing biofilm of plastic surface, the biofilm was challenged for 6 h with varying concentration of lemongrass essential oil in emulsion and microemulsion form. MBEC assay were determined when 50% of biofilm was eradicated. As depicted in Fig. 3, the minimum concentration of lemongrass essential oil for biofilm eradication was 1% (v/v) for both emulsion and microemulsion, with eradication percentage being 53.03 and 88.93%, respectively.

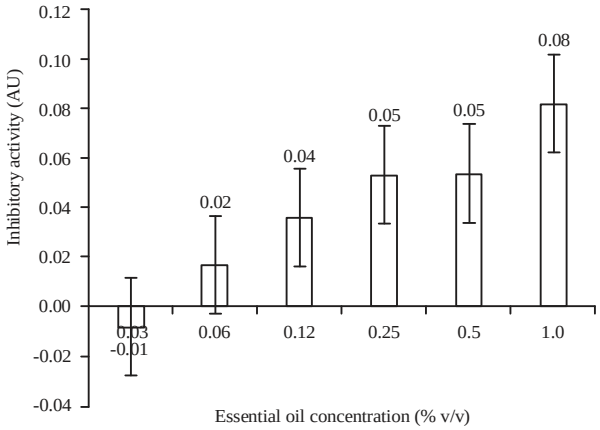

Fig. 1: Inhibitory activity of lemongrass essential oil emulsion against the growth of planktonic biofilm-forming bacteria. Result showed that minimum concentration of lemongrass essential oil emulsion needed to inhibit cell growth is 0.06% (v/v), and increasing concentration of essential oil would improve its inhibitory activity

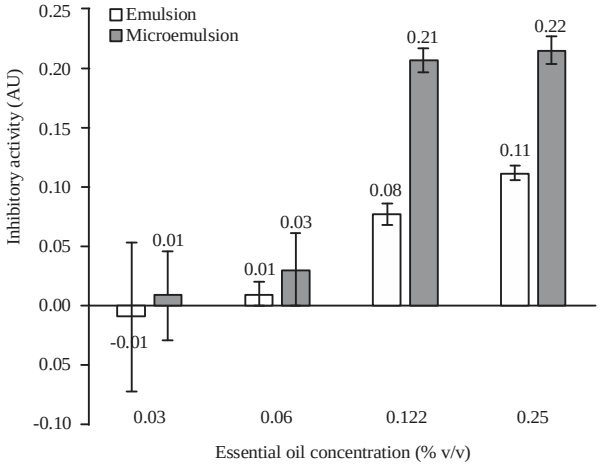

Fig. 2: Inhibitory activity of lemongrass essential oil emulsion and microemulsion against biofilm formation. Microemulsion exhibited higher inhibitory activity compared with emulsion. MBIC of emulsion and microemulsion were 0.06 and 0.03%, respectively

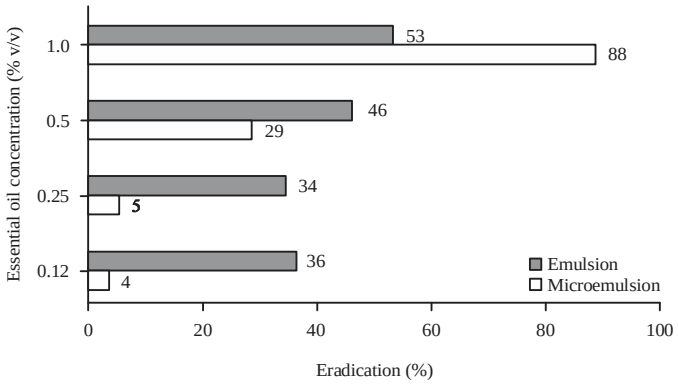

Fig. 3: Biofilm eradication by lemongrass essential oil emulsion and microemulsion. $MBEC_{50\%}$ of both emulsion and microemulsion were 1%

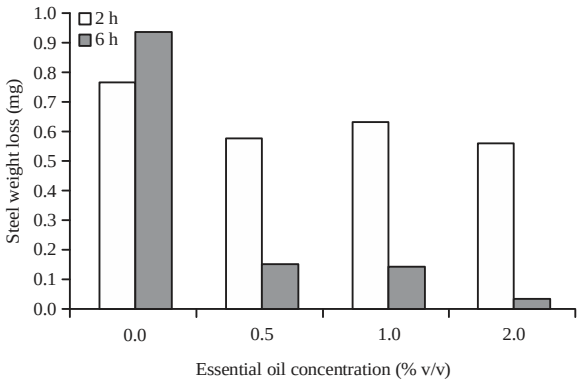

Fig. 4: Biocorrosion in formation water exposed with lemongrass essential oil for 2 and 6 h. Exposure of 2% essential oil for 6 h resulted in the highest steel weight loss

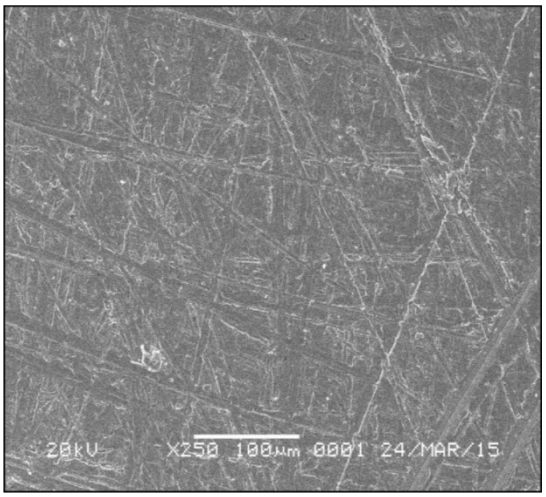

Fig. 5: Scanning electron microscope image of steel surface. The image shows the clean surface of ST-37 steel coupon prior to biofilm or biocorrosion development

Corrosion rate measurement: Corrosion inhibition effect of essential oil was investigated by analyzing the weight loss of biofilm-infested steel coupons, as weight loss indicates biocorrosion occurrence. After 6 or 2 h of essential oil exposure in varying concentration, coupons were cleaned and weighed. The data in Fig. 4 showed the resulting weight loss in each treatment. The calculated corrosion rate for 0, 0.5, 1 and 2% LEO treatment were 25.43, 19.07, 21 and 18.57 mm/year with 2 h of exposure period and 30.96, 4.97, 4.64 and 0.99 mm/year with 6 h of exposure period. Lowest corrosion rate, e.g., 0.99 mm/year, was observed at the treatment of 2% lemongrass essential oil exposure in emulsion form for 6 h.

Inhibitor efficiency (IE): Inhibitor efficiency (IE) indicated the efficiency of biocide agent in mitigating biofilm-biocorrosion based on weight loss calculation. Inhibitor efficiency of 0.5, 1 and 2% LEO treatment were 25, 17.39 and 26.96% with 2 h of exposure period and 83.93, 84.99 and 96.79% with 6 h of exposure period. The highest IE, e.g., 96.79%, was obtained from the treatment of 2% lemon grass essential oil for 6 h.

Biofilm-biocorrosion morphological observation: Energy dispersive X-ray (EDX) spectroscopy analyzed the metal composition of ST-37 carbon steel coupons (Table 1). The result indicated that ST-37 carbon steel coupons were suitable for biofilm growth. The image of steel coupons surface as observed with scanning electron microscope prior to biofilm formation was showed in Fig. 5. Coupon's surface appeared clean and microbial cells nor EPS were not observed. The results in Fig. 6a and b provided further enlarged images of 14 days biofilm formed on steel surface, 500× and 2000×, respectively. The surface of metal after biofilm was treated with 2% of essential oil for 6 h was showed in Fig 6, whereas, Fig. 6d showed damages on steel surface that was inflicted by biocorrosion.

DISCUSSION

Main component of lemongrass essential oil is citral, constituting up to 78.2%. Citral, a compound known to possess antimicrobial activity, is monoterpene an aldehyde which could disrupt microbial cell membrane stability and cause membrane leakage[16]. This study aimed to provide better understanding of lemongrass essential oil potential in inhibiting and eradicating biofilm formation, specifically those found in formation water environment. These environments were commonly found in oil industry pipeline system, which is prone to biocorrosion that leads to shorter lifetime of metal pipes.

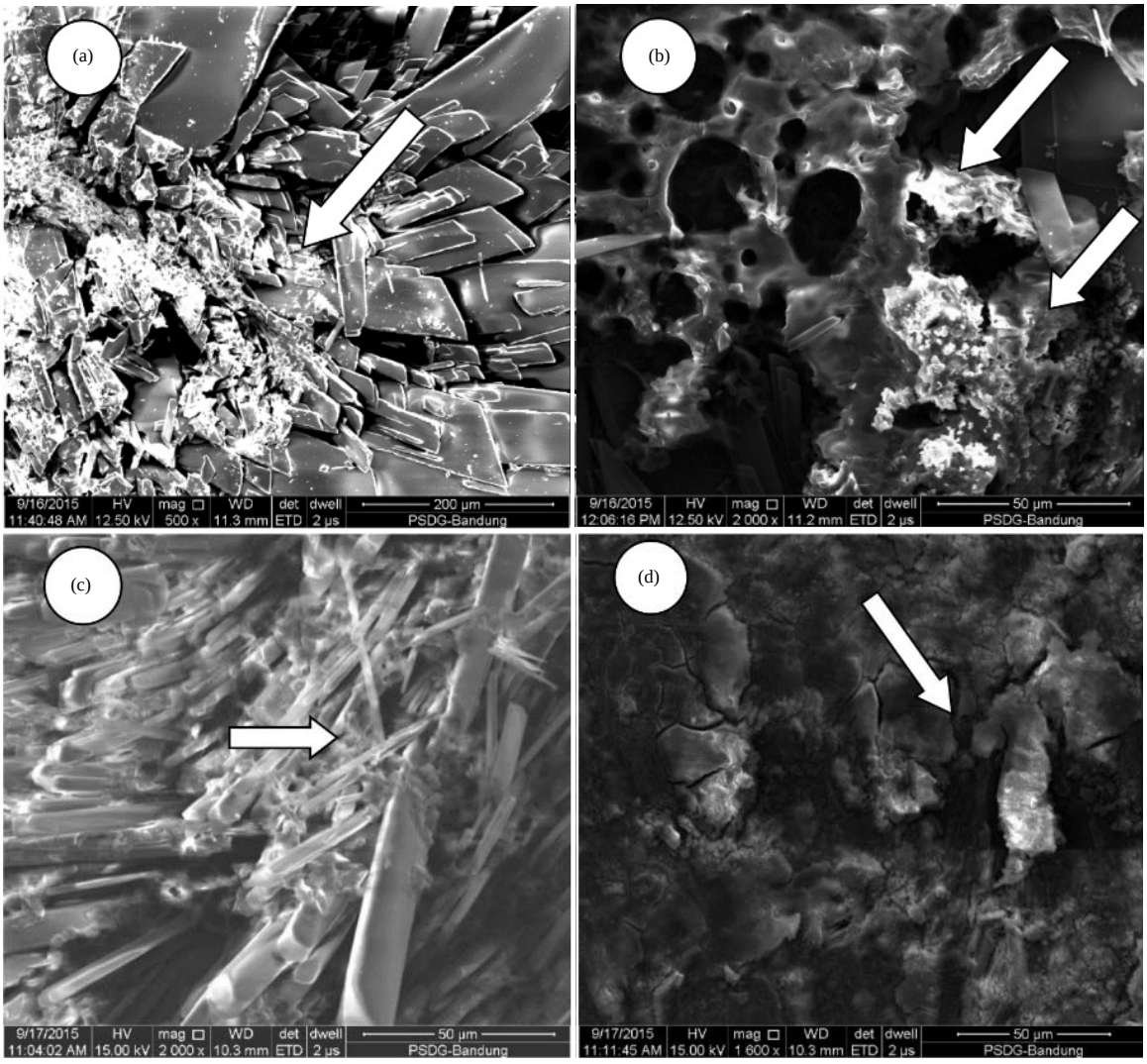

Fig. 6(a-d): Scanning electron microscope image of biofilm-infested steel surface taken before and after exposure of essential oil, (a and b) Biofilm was observed on steel surface after 14 days of incubation with microbial culture, (c) Biofilm structure was damaged after treatment with essential oil microemulsion and (d) Corrosion inflicted in steel surface after biofilm development, white arrow indicate damage on steel surface. White arrows on a, b, c and d point to EPS or microorganism on biofilm

Table 1: The ST-37 carbon steel composition

	Metal composition based on EDX analysis					
Elements	Fe	Mo	Mn	Al	C	O
Abundance (%)	75.90	0.26	0.22	0.16	0.08	22.58
Compounds	FeO	MoO_3	MnO	Al_2O_3	C	P_2O_5
Abundance (%)	97.64	0.39	0.28	0.30	0.08	0.20

This study also explored the essential oil in two different form, emulsion and microemulsion. The availability of active compounds also depends on its physical form in the environment. This study challenged the biofilm-biocorrosion inducing microorganism found in formation water with two different form of lemongrass essential oil. Initial experiment in determining inhibitory activity of oil emulsion toward planktonic microbial cells showed its minimum inhibitory concentration (MIC) is 0.06% (Fig. 1).

Present study also showed that lemongrass essential oil could inhibit biofilm formation. The minimum biofilm inhibitory concentration (MBIC) assay showed that lemongrass essential oil concentration in emulsion form needed to inhibit biofilm formation was 0.06% (v/v) which was equivalent to MIC concentration. This was because biofilm-formation inhibition is proportional to planktonic cell growth inhibition, since biofilm differentiation depends on proliferation of planktonic cells. However, the assay showed that similar impact can be achieved using lower concentration of lemongrass essential oil in microemulsion form (0.03%) (Fig. 3). This result proved that formulating essential oil into microemulsion would increase its solubility, dissolution degree and bioavailability to interact with planktonic bacterial cell as well as biofilm[11].

Biofilm is a microbial community structure commonly formed in unfavorable environment[17]. Within this physical structure consisting cells and extracellular polymeric substance matrix, microorganism's survivability towards antibiotic compound increases up to 1000×, compared to that in planktonic form. As minimum biofilm eradication concentration (MBEC) assay showed, emulsion and microemulsion of essential oil disrupted biofilm to varying degree, even at the lowest tested concentration of 0.12% (Fig. 3). It was known that citral in essential oil is capable to impair cell wall, react with polysaccharides in EPS matrix and damage cell membrane's stability. The results in Fig. 3 also showed that 1% of essential oil in microemulsion have greater impact in eradicating biofilm. However, in other range of concentration, some irregularities were observed. Particle size analysis of microemulsion showed that the particle size was unevenly distributed. This might have contributed to lower eradication at lower concentration, compared to that of emulsion. This result indicated that further optimization is needed in formulating microemulsion to produce more uniform distribution of particle size.

SEM-EDX analysis showed that carbon steel coupons used in this study contained low carbon constituent (0.08%), an appropriate characteristic to the commonly used industrial internal pipes. Fe and FeO which constitute the main part of metal were suitable to support biofilm-biocorrosion growth[12]. Previous study showed that anaerobic bacteria and aerobic bacteria were detected on steel plate surface after 14 days of incubation in formation water in the amount of log 9.083 and log 3.6.35 CFU mL^{-1}, respectively[12].

Biofilm-biocorrosion inhibition potential assay did not include the use of essential oil in microemulsion form due to uneven distribution of particle size as previously described. The assay assessed the potential of lemongrass essential oil emulsion in eradicating biofilm-biocorrosion on carbon steel coupons, in varying concentration and two variation of exposure time, 2 and 6 h. These exposure periods were chosen according to common industrial practice of biofilm-biocorrosion mitigation with biocides treatment, which is 6 h. From the experiment, it was observed that by exposing the metal to higher concentration of lemongrass essential oil (2%) and for longer time (6 h) resulted in lower weight loss (Fig. 4). On those corroded steel coupons, X-ray diffraction (XRD) analysis observed the presence of vivianite and hematite crystal deposit on its surface, hematite crystal was known to promote further corrosion[12].

The SEM analysis revealed that biofilm-biocorrosion could form on carbon steel that has been incubated in formation water. Following lemongrass essential oil emulsion exposure, biofilm appeared to be thinner and less compact compared to the untreated biofilm. This result indicated that essential oil was able to disrupt biofilm, including the cells and EPS matrix. After removing the biofilm on the metal surface, corrosion was evident in areas where accumulated microbes were found. Microbial settlement on the surface was known to produce acid or gas that promote the corrosion[12]. Thus, inhibited biofilm formation would also inhibit biocorrosion it would potentially trigger.

CONCLUSION

Based on MIC, MBIC and MBEC assays, lemongrass essential oil showed the ability to inhibit biofilm formation and prevent biocorrosion. Results concluded that essential oil emulsion and microemulsion can serve as alternative to chemical biocide in biofilm-biocorrosion mitigation. In addition, lemongrass essential oil in microemulsion showed better inhibitory effect towards biofilm formation. However, microemulsion formulation still requires further optimization.

SIGNIFICANCE STATEMENT

This research aimed to study the potential of lemongrass essential oil to mitigate biofilm-biocorrosion in formation water environment. This study discovered the potential of natural plant extract as a source of active compound that has antagonistic activity towards biofilm progression that can be beneficial to improve the process of biocorrosion mitigation. This study will serve as a preliminary study for other researchers to reveal further findings regarding biofilm, biocorrosion and ways to overcome it.

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REFERENCES

1. Jonan, I., S. Nugraha, E. Prasodjo, H. Nurzaman and Walujanto *et al*., 2016. Indonesia energy outlook 2016. National Energy Council, Jakarta, pp: 16-19.
2. Al-Sayegh, A., Y. Al-Wahaibi, S. Al-Bahry, A. Elshafie, A. Al-Bemani and S. Joshi, 2015. Microbial enhanced heavy crude oil recovery through biodegradation using bacterial isolates from an Omani oil field. Microbial Cell Factories, Vol. 14. 10.1186/s12934-015-0330-5.
3. Augustinovic, Z., O. Birketveit, K. Clements, M. Freeman and S. Gopi *et al*., 2012. Microbes-oilfield enemies or allies? Oilfield Rev., 24: 4-17.
4. Da Rosa, J.P., S.R.G. Tiburcio, J.M. Marques, L. Seldin and R.R.R. Coelho, 2016. *Streptomyces lunalinharesii* 235 prevents the formation of a sulfate-reducing bacterial biofilm. Braz. J. Microbiol., 47: 603-609.
5. Koch, G.H., M.P. Brongers, N.G. Thompson, Y.P. Virmani and J.H. Payer, 2002. Corrosion cost and preventive strategies in the United States. FHWA-RD-01-156, NACE International, Ohio, pp: E5.
6. Bhola, S.M., F.M. Alabbas, R. Bhola, J.R. Spear, B. Mishra, D.L. Olson and A.E. Kakpovbia, 2014. Neem extract as an inhibitor for biocorrosion influenced by sulfate reducing bacteria: A preliminary investigation. Eng. Failure Anal., 36: 92-103.
7. Hammer, K.A., C.F. Carson and T.V. Riley, 1998. *In-vitro* activity of essential oils, in particular *Melaleuca alternifolia* (tea tree) oil and tea tree oil products, against *Candida* spp. J. Antimicrob. Chemother., 42: 591-595.
8. Sofiah, S., 2010. *Cymbopogon citratus* sebagai salah satu tanaman penghasil minyak atsiri. UPT Balai Konservasi Tumbuhan Kebun Raya, Indonesia.
9. Korenblum, E., F.R. de Vasconcelos Goulart, I. de Almeida Rodrigues, F. Abreu and U. Lins *et al*., 2013. Antimicrobial action and anti-corrosion effect against sulfate reducing bacteria by lemongrass (*Cymbopogon citratus*) essential oil and its major component, the citral. AMB Express, Vol. 3. 10.1186/2191-0855-3-44.
10. Adukwu, E.C., S.C.H. Allen and C.A. Phillips, 2012. The anti-biofilm activity of lemongrass (*Cymbopogon flexuosus*) and grapefruit (*Citrus paradisi*) essential oils against five strains of *Staphylococcus aureus*. J. Applied Microbiol., 113: 1217-1227.
11. Anwer, M.K., S. Jamil, E.S. Ibnouf and F. Shakeel, 2014. Enhanced antibacterial effects of clove essential oil by nanoemulsion. J. Oleo Sci., 63: 347-354.
12. Wang, X., J. Xu and C. Sun, 2013. Effects of sulfate-reducing bacterial on corrosion of 403 stainless steel in soils containing chloride ions. Int. J. Electrochem. Sci., 8: 821-830.
13. Bolton, N., M. Critchley, R. Fabien, N. Cromar and H. Fallowfield, 2010. Microbially influenced corrosion of galvanized steel pipes in aerobic water systems. J. Applied Microbiol., 109: 239-247.
14. Meduri, C.S., 2014. Corrosion resistance and mechanical properties of amorphous metal honeycombs in NaCl environments. M.Sc. Thesis, Oklahoma State University, Oklahoma.
15. Hussin, M.H. and M.J. Kassim, 2011. The corrosion inhibition and adsorption behavior of *Uncaria gambir* extract on mild steel in 1M HCl. Mater. Chem. Phys., 125: 461-468.
16. Tyagi, A.K. and A. Malik, 2010. Liquid and vapour-phase antifungal activities of selected essential oils against *Candida albicans*: Microscopic observations and chemical characterization of *Cymbopogon citratus*. BMC Complement. Altern. Med., Vol. 10. 10.1186/1472-6882-10-65.
17. Yarwood, J.M., D.J. Bartels, E.M. Volper and E.P. Greenberg, 2004. Quorum sensing in *Staphylococcus aureus* biofilms. J. Bacteriol., 186: 1838-1850.