

Biosurfactant Produced by Indigenous Bacteria During Composting Process of Crude Oil Polluted Soil: Properties and Role

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ABSTRACT

Bacterial co-metabolism in composting process has been widely used to remove hydrocarbons, aided by in-situ production of bio-based surfactants, in terms of compost humic acid-like substances and biosurfactants. The properties of compost humic acid-like substances have been shown in previous studies as potential surface tension reducers and emulsifiers for hydrocarbons. The current study aimed to analyze the properties of biosurfactant of surface tension decrease, emulsification activity, and hydrocarbon solubilization ability. Four indigenous bacteria consortia were isolated from composted materials of yard waste, rumen residue, crude oil-polluted soil, and the mixture of polluted soil with organic waste (1:1, w/w) at day 0th, 20th, 40th, and 60th. Organic waste consists of yard waste and rumen residue in the ratio of 1:1. The isolated indigenous bacteria consortia were incubated for 7 days in different media, i.e., organic waste extract, 6.00% of crude oil, and a mixture of organic waste extract with 6.00% crude oil. The results indicated that the surface tension decrease and emulsification activity of biosurfactants were 8.35–52.90 mN m⁻¹ and 0.00–12.00%, respectively, which showed the potential as surface tension reducers with low emulsification activity. The higher hydrocarbon solubility was shown by the biosurfactant from the rumen residue (13 620 µg g⁻¹) and the mixture (10 998 µg g⁻¹) at day 40th, which was comparable to 1.50% of Tween 80. The biosurfactants in the current research were produced with the same materials, process, and time as compost humic acid-like substances which acts as in-situ bio-based surfactants. The respective ability to solubilize hydrocarbon might be combined and estimated to be higher than Tween 80 of 24 329 µg g⁻¹ and 21 619 µg g⁻¹ for rumen residue and the mixture, respectively. Therefore, it was concluded that the best composition for in-situ bio-based surfactant production to assist the degradation of hydrocarbon through composting process is polluted soil with organic waste (1:1, w/w). The solubility of hydrocarbons can be increased without synthetic surfactants addition, but through providing nutrients to maintain in-situ bio-based surfactant production with intermittent addition of organic waste every 40 days. This method is expected to be an appropriate approach in composting development as a cost-effective sustainable bioremediation technique for polluted soil.

Keywords: bioremediation; biosurfactant; composting; hydrocarbon; in-situ bio-based surfactant; polluted soil.

INTRODUCTION

Petroleum hydrocarbons are one of the hazardous contaminants (Agency for Toxic Substances and Disease Registry, 1999; Bamforth and Singleton, 2005) that widely pollute soil in many oil-producing countries (Bamforth and Singleton, 2005; Sari et al., 2018b, 2018a). Petroleum hydrocarbons are hydrophobic, which makes it easy to bind organic materials and form micropollutants in the soil (Bamforth and Singleton, 2005). Therefore, it causes that petroleum hydrocarbons are hard to reach and degrade by bacteria, becoming more dangerous and toxic for plants, organisms, humans, and environment (Bamforth and Singleton, 2005; Sari et al., 2018a, 2018b), possibly leading to gene mutation (Bamforth and Singleton, 2005; Jiménez-Peñalver et al., 2019; Torres et al., 2005).

Composting has been studied as an alternative cost-effective method for removing petroleum hydrocarbons from polluted soil. Petroleum hydrocarbon removal efficiency in the composting process ranges from 61.20% to 96.00% (Hesnawi and Mogadami, 2013; Trihadiningrum et al., 2018). Composting was conducted by mixing the petroleum hydrocarbon polluted soil with organic waste amendments as the main nutrient and exogenous bacteria sources (Sari et al., 2019; Trihadiningrum et al., 2018). Organic waste amendments are utilized to stimulate hydrocarbonoclastic bacteria activities, which lead to the increase of petroleum hydrocarbon biodegradation efficiency (Trihadiningrum et al., 2018; Zhang et al., 2012). Our previous study also reported that the composting process using yard waste and rumen residue increases the degradation of petroleum hydrocarbons in crude oil-polluted soil by 31 times compared to untreated soil over 120 days (Sari et al., 2019). It might be supported by in-situ bio-based surfactants which are formed during composting process (Kulikowska et al., 2015; Sari et al., 2018c).

In-situ bio-based surfactants, indicated as amphiphilic compounds, potentially act like synthetic surfactant that are able to decrease surface tension and increase emulsification and desorb petroleum hydrocarbon from soil (Mulligan, 2021). Therefore, bio-based surfactants have lower toxicity; in addition, they are and more biodegradable and environment-friendly than synthetic surfactants (Mulligan, 2021; Ni'matuzahroh et al., 2016). In-situ bio-based surfactants are divided

into compost humic acid like substances (cHAL) and biosurfactants which are formed during organic waste decomposition by many indigenous bacteria in composting process. Our previous study reported that cHAL obtained from crude oil-polluted soil composting process using yard waste and rumen residue on day 40th was able to solubilize petroleum hydrocarbons and reduce surface tension by 10,621 $\mu\text{g g}^{-1}$ and 21.65 mN m^{-1} , respectively (Sari et al., 2018c). Biosurfactants are natural extracellular compounds produced by bacteria through cell biotransformation (Jahanshah et al., 2013). They have received great attention for their applications in treating hydrocarbon-contaminated soils (Mulligan, 2021; Zhou et al., 2013). Desorbed hydrocarbons which become soluble facilitate their bio-availability and bio-compatibility for microbes in an optimal moisture, 50–60% (Jahanshah et al., 2013; Zhou et al., 2013). To the best of our knowledge, the biosurfactants used to enhance the solubility and bioavailability of pollutants are produced by exogenous bacteria and added to the bioremediation process (Zhou et al., 2013). The production can be quite complicated, because it uses specific bacteria that have been isolated and enhanced. This process is considered inefficient, because it does not empower the potential of indigenous bacteria in the process. The composting process of a crude oil-polluted soil organic waste mixture, which is part of this current research and previously reported by Sari et al. (2019), found *Bacillus sp.*, and *B. cereus* as the dominant bacteria that are confirmed as hydrocarbonoclastic bacteria (Cerqueira et al., 2011). In addition, the bacteria belonging to the genus *Bacillus* have the potential as biosurfactant producers (Bezza and Chirwa, 2015; Heryani and Putra, 2017; Jahanshah et al., 2013; Sakthipriya et al., 2015).

However, the performance of these bacteria with others that are incorporated as a consortium of indigenous bacteria in crude oil composting process in producing biosurfactants has not been clearly identified. Biosurfactant production by consortium of indigenous bacteria during composting process has become the focus at this current research. The aims of this research were to determine the properties of biosurfactant produced by indigenous bacteria consortium for reducing surface tension, emulsifying and solubilizing hydrocarbon from crude oil-polluted soil. The properties were compared to Tween 80, a widely used synthetic surfactant. The authors also tried to

carry out a comprehensive investigation of cHAL and biosurfactant role as in-situ bio-based surfactant in the crude oil composting process. This current study is an approach to optimize composting as a cost-effective method without a synthetic surfactant or other external biosurfactant addition for crude oil-polluted soil bioremediation.

MATERIALS AND METHODS

Preparation of bacterial solution suspension

Isolation of indigenous bacteria as a consortium was prepared from different composted materials and periods for the composting process. The composted materials consisted of yard waste, rumen residue, soil polluted with crude oil 6.05% and the mixture of crude oil-polluted soil and organic waste at a 1:1 ratio (w/w). Five g (5.00) of the sample were taken on day 0th, 20th, 40th, and 60th from each composted material, then dissolved into 45.00 mL of 0.90% NaCl (Merck 1.06404) and agitated using a shaker at 100 rpm for 24 hours to obtain a bacterial solution suspension (Pratiwi, 2012). In the current research, the types of specific bacteria included in the consortium of each composted material were not characterized to encourage the performance of indigenous bacteria without adjustment and control. Following Pratiwi (Pratiwi, 2012) and Ni'matuzahroh et al. (2016), as much as 4.00% of the bacterial suspension was inoculated in 96.00% of growth medium.

Preparation of growth medium for bacteria

The growth media for bacteria in this study were prepared from 3 extracts, in terms of organic waste (a mixture of yard waste and rumen residue in the ratio of 1:1, w/w), crude oil, and a mixture of crude oil-polluted soil and organic waste (1:1, w/w). The extract was prepared to represent the existing nutrients of bacteria in crude oil-polluted soil composting process.

Organic waste extract

Yard waste and rumen residue (1:1, w/w) as organic waste were extracted by modified cold percolation and solvent extraction methods [11, 21]. Before extraction, organic waste was sterilized to eliminate bacteria using an autoclave at 121.00°C and 1.00 atm for 20 minutes. Ten g

(10.00 g) of sterilized organic waste were mashed and soaked with 40.00 mL distilled water, then agitated for 2 hours at 100 rpm using rotary shaker to dissolve polar compounds. Furthermore, it was decanted to separate the liquid phase. The solid phase was soaked using 50.00 mL of 1.00% NaOH (Merck 1.06498) to dissolve lignocellulose and then separated using Whatman 42 filter paper. These separation procedures were repeated three times. Both liquid phases were mixed and evaporated at 90.00°C for 10 hours to obtain an organic waste extract.

Crude oil

Crude oil was taken from Wonocolo public oilfields in Bojonegoro, East Java, Indonesia, which acts as a carbon source for bacteria. As much as 6.00 mL of crude oil were added to 90.00 mL of mineral salt medium (MSM) to meet the requirement of 96.00 mL (96.00%) of bacterial growth media, as described in subsection 2.1. The volume of crude oil used is intended to represent the existing soil pollution in the Wonocolo public oilfields, as reported by Sari et al. (2018b, 2018a). MSM is made by dissolving (NH₄)₂SO₄ (3.00 g), MgSO₄·7H₂O (0.20 g), NaCl (10.00 g), CaCl₂ (0.01 g), MnSO₄·H₂O (0.001 g), H₃BO₃ (0.001 g), and ZnSO₄·7H₂O (0.001 g) in 1000 mL of distilled water using a magnetic stirrer. The pH of solution was neutralized by 10.00% NaOH (Merck 1.06498) or 5.00% HCl (Merck 1.00317) addition and placed into a 500 mL tube, which is known as a macro nutrient solution. The micro-nutrient solution was prepared from KH₂PO₄ (1.00 g) and FeSO₄·7H₂O (1.00 g) added to 50.00 mL distilled water, respectively, and then was sterilized and mixed (Pruthi and Cameotra, 1997).

A mixture of organic waste extract and crude oil

Organic waste extract and crude oil were mixed to simulate the existing conditions of a polluted soil composting process with a volume of 90.00 mL and 6.00 mL, respectively. It is similar to crude oil as growth media.

Production and extraction of biosurfactant

The production of biosurfactant was carried out using the mixture of bacterial suspension and growth media (listed in Table 1). Each mixture

Table 1. Indigenous bacterial solution suspension and growth media for biosurfactant production

No	Indigenous bacterial solution suspension (4.00%)	Bacterial growth media (96.00%)	Code
1.	4.00 mL of bacterial suspension from a mixed of crude oil polluted soil with organic waste (yard waste and rumen waste) at 1:1 ratio (w/w)	96.00 mL of organic waste extract	SW _o
		96.00 mL of crude oil and MSM mixture	SW _c
		96.00 mL of organic waste extract and crude oil mixture	SW _{oc}
2.	4.00 mL of bacterial suspension from yard waste	96.00 mL of organic waste extract	Y _o
		96.00 mL of crude oil and MSM mixture	Y _c
		96.00 mL of organic waste extract and crude oil mixture	Y _{oc}
3.	4.00 mL of bacterial suspension from rumen waste	96.00 mL of organic waste extract	R _o
		96.00 mL of crude oil and MSM mixture	R _c
		96.00 mL of organic waste extract and crude oil mixture	R _{oc}
4.	4.00 mL of bacterial suspension from crude oil polluted soil	96.00 mL of organic waste extract	S _o
		96.00 mL of crude oil and MSM mixture	S _c
		96.00 mL of organic waste extract and crude oil mixture	S _{oc}
5.	Synthetic surfactant	Tween 80 (1.50%)	Tween 80 (1.50%)

was agitated using a shaker at 120 rpm for 7 days to produce biosurfactant (Pratiwi, 2012), then separated from the media using a centrifuge at 4000 rpm for 30 minutes (Sharma et al., 2001). The produced biosurfactant was stored at 4.00°C to avoid degradation (Pratiwi, 2012; Sharma et al., 2001). The biosurfactant production process was performed in duplicate.

Measurement of surface tension decrease

Surface tension decrease (ΔST) is the difference in surface tension (ST) value between surfactant (biosurfactant or synthetic surfactant) and distilled water. The measurement of ΔST was conducted using a Du Nouy Tensiometer (K6, Krüss-scientific, Germany) and calculated according to the formula of ASTM D 1331 (ASTM D 1331, 2000):

$$r = (\Theta / \Theta_0) \times r_0 \quad (1)$$

where:

r = the ST of biosurfactant

r_0 = the ST of distilled water

Θ = the scale result of biosurfactant measurement using Du Nouy tensiometer

Θ_0 = the scale result of distilled water measurement using Du Nouy tensiometer

Measurement of emulsification activity

Emulsification activity (EA) was measured by mixing a biosurfactant and kerosene in the ratio of 1:1 (v/v) using vortex mixer and left to

stabilize for 3 minutes and 24 hours, respectively (Pruthi and Cameotra, 1997). The EA was determined by emulsification index using the formula:

$$EA (\%) = \frac{\text{emulsion height (cm)}}{\text{total solution height (cm)}} \times 100\% \quad (2)$$

Measurement of biosurfactant ability to solubilize hydrocarbon

The ability of biosurfactant to solubilize hydrocarbons from polluted soil was carried out in several steps, in terms of soil washing, soil extraction, and hydrocarbon analysis. Following Jatnika and Hadrah (Jatnika and Hadrah, 2015), soil washing was conducted by mixing and agitating the polluted soil and biosurfactant in the ratio of 1:10 (w/v) using a rotary agitator at 200 rpm in batch conditions. These processes were performed for 20 hours at room temperature (29–33°C), then the samples were separated using a Whatman No. 42 filter to obtain the soil residue. The soil residue was extracted using Soxhlet and Gravimetric method to obtain hydrocarbon extract, which follows the standards of APHA-AWWA-WEF (APHA-AWWA-WEF, 2005) No. 5520D and 5520F, respectively. The analysis of the hydrocarbons extract was carried out by FT-IR Spectrometer at 2930 cm⁻¹ of absorbance, following the ASTM standard No. D7066-04 (ASTM D7066-04, 2011). Furthermore, the measurement of the biosurfactant ability to solubilize hydrocarbons was calculated by the differences in hydrocarbon levels in polluted soils during the soil washing process. Those steps were also

carried out for the commercial surfactant, Tween 80 at a 1.50% concentration which shows the optimal value for hydrocarbon solubilization Sari et al. (2018c), as a control in current research. The tests for all samples were performed in duplicate.

RESULTS AND DISCUSSION

Bacterial consortium growth during incubation period

The growth of the isolated indigenous bacterial consortium mostly increased during the 7-day incubation period, especially on day 60th of composting process, where the highest was found in SW_{oc} of 7.60x10¹⁶ CFU mL⁻¹ (Fig. 1). It shows

that the appropriate combination and composition between crude oil polluted soil and the mixture of yard waste and rumen residue as organic waste could serve as sufficient nutrients for indigenous bacteria consortium in composting process (Sari et al., 2019; Trihadiningrum et al., 2018). The indigenous bacterial consortium at SW_{oc} utilizes biodegradable nutrients from organic waste that stimulates the growth while encouraging petroleum hydrocarbons that become more bioavailable. It allows indigenous bacteria to adapt to the presence of petroleum hydrocarbons in polluted soil and utilize it as co-nutrients (Cerqueira et al., 2011; Sari et al., 2019; Trihadiningrum et al., 2018). The decomposition of hydrocarbons takes longer than other organic materials due to

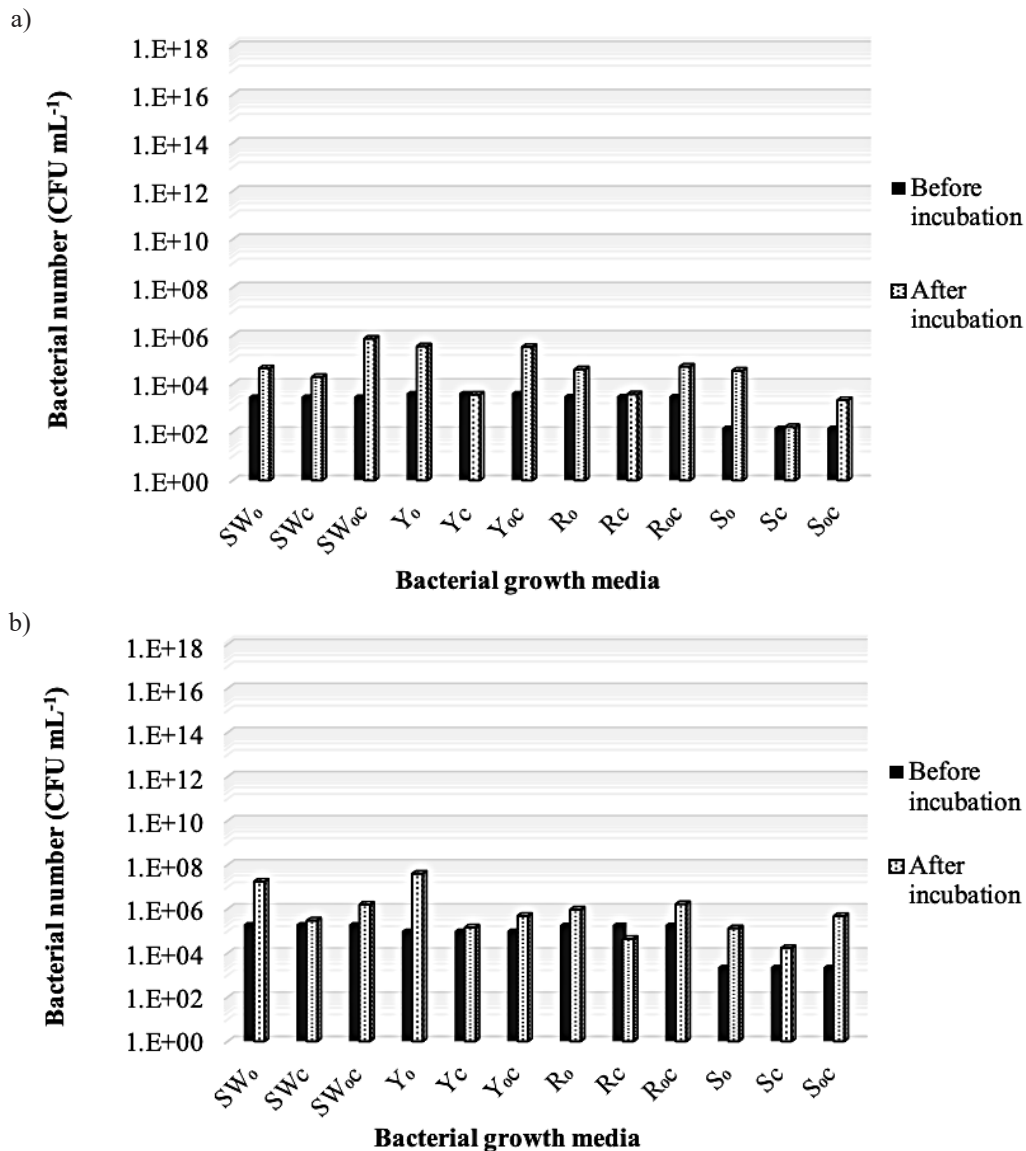


Figure 1. Isolated bacterial growth during 7-days incubation period: (a) Day 0; (b) Day 20; (c) Day 40; (d) Day 60

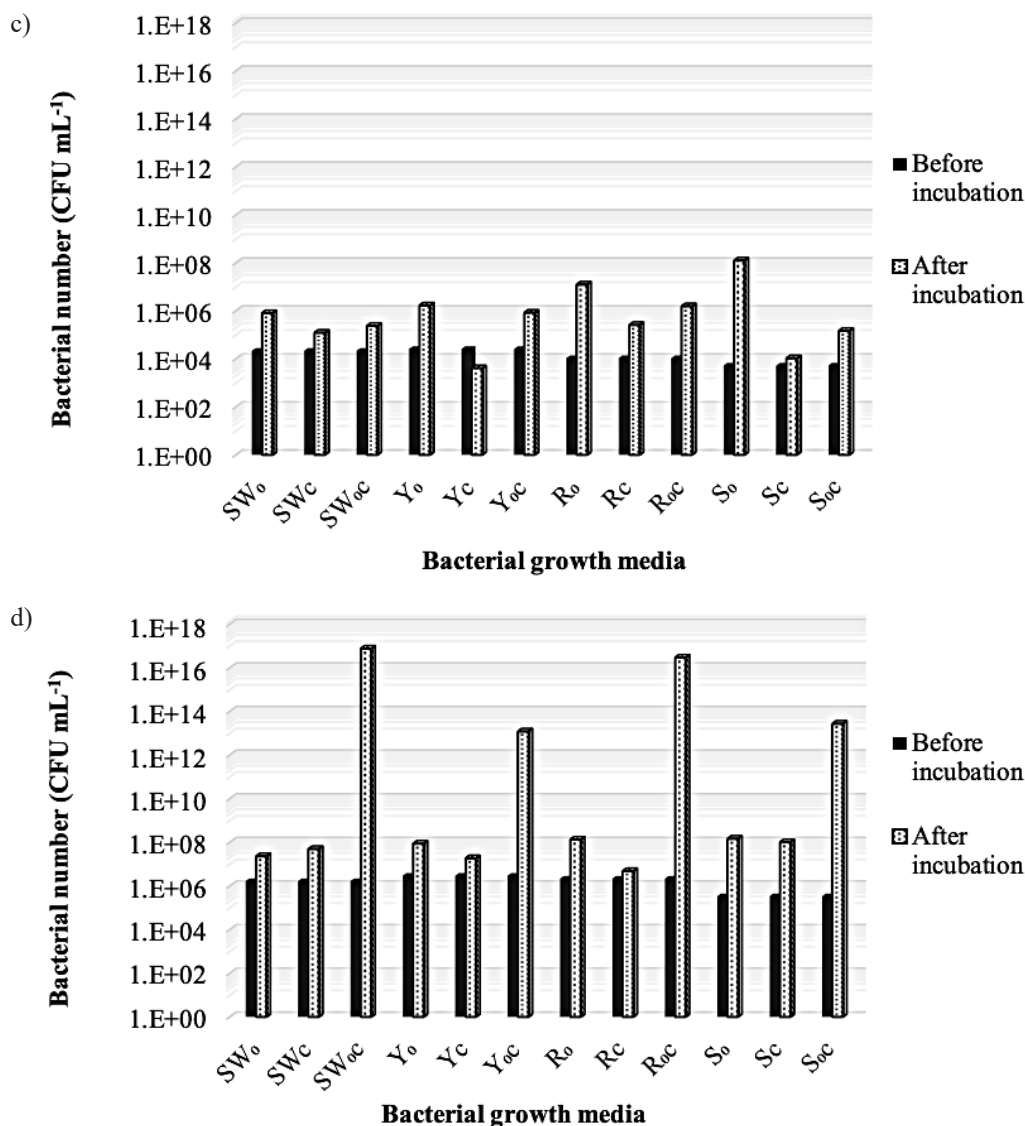


Figure 1c, 1d. Cont. Isolated bacterial growth during 7-days incubation period: (a) Day 0; (b) Day 20; (c) Day 40; (d) Day 60

its complex structure, as explained by Pruthi and Cameotra (1997), as different carbon sources will affect the growth and reproduction of bacteria. It may be indicated by varying pH values during incubation period, which ranges from 3.00 to 13.00 (Fig. 2). The pH values of SW_{oc} from day 40th and 60th, ranged between 6.00–12.00 during incubation period, which are the preferred conditions for bacterial growth and biosurfactant production (Raza et al., 2007; Sayara et al., 2010).

After 7 days of incubation period, Fig. 2 also shows that the lowest pH value was found in the crude oil media for those of bacterial solution, ranging from 2.00 up to 5.00. It indicates that the acidification process still occurs, where hydrocarbons become the main nutrients for bacteria

and decomposed into volatile acids (Sayara et al., 2010). It causes the growth to be disrupted, which was followed by lower bacterial growth compared to other bacterial solutions concentrations of 1.45×10^2 – 1.01×10^8 CFU mL⁻¹. However, the dynamics of changes in the number of colonies formed indicates that the presence of 6.00% crude oil does not always have a toxic effect for bacteria.

Comparison of surface tension decrease between biosurfactant and Tween 80 (1.50%)

The Δ ST generated by biosurfactants from each experiment showed varied values (Fig. 3), where the lowest and the highest were found in S_c day 20th and SW_{oc} day 40th of 8.35 mN m⁻¹ and

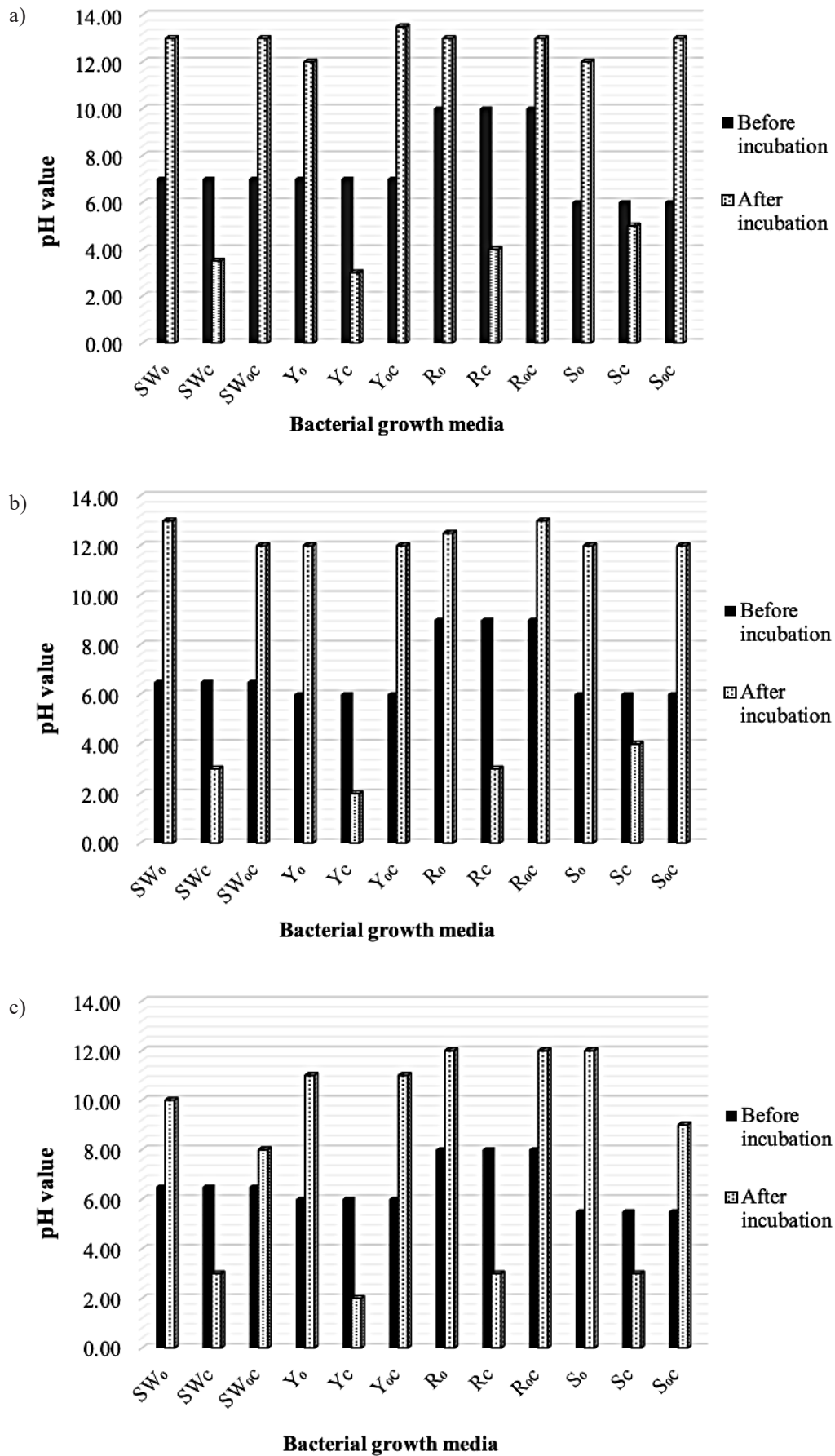


Figure 2. The changes of pH value during incubation period: (a) Day 0; (b) Day 20; (c) Day 40; (d) Day 60

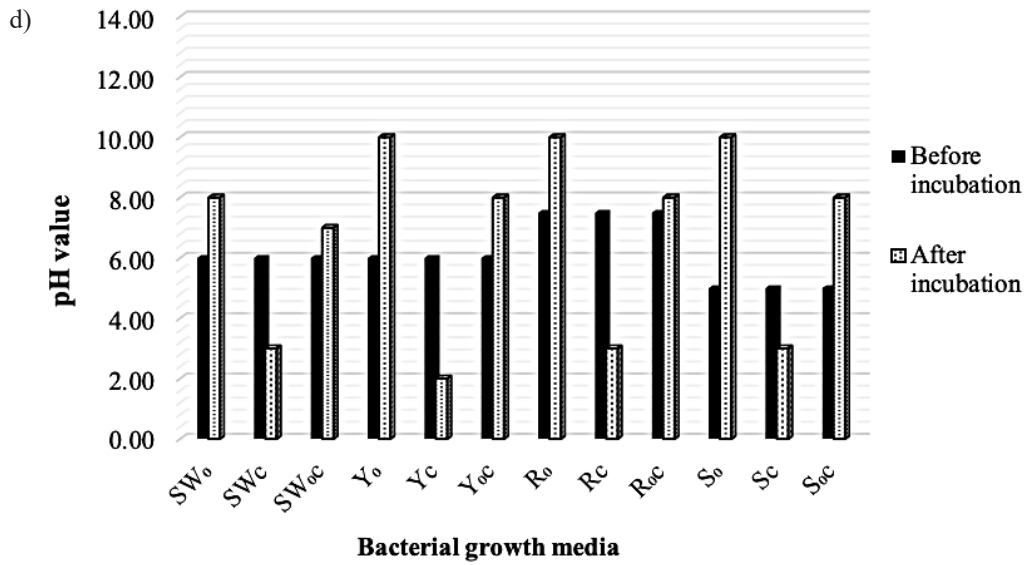


Figure 2d. Cont. The changes of pH value during incubation period: (a) Day 0; (b) Day 20; (c) Day 40; (d) Day 60

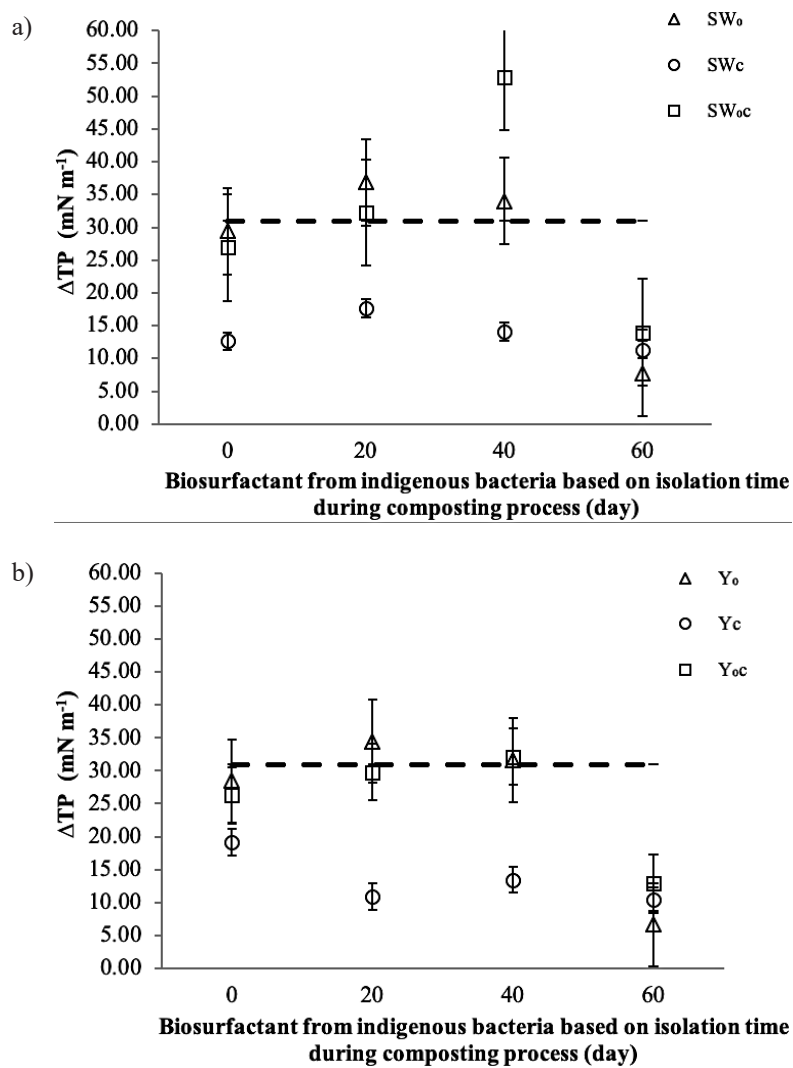


Figure 3. Surface tension decrease of biosurfactants: (a) Mixed crude oil polluted soil with organic waste; (b) Yard waste; (c) Rumen residue; (d) Crude oil polluted soil

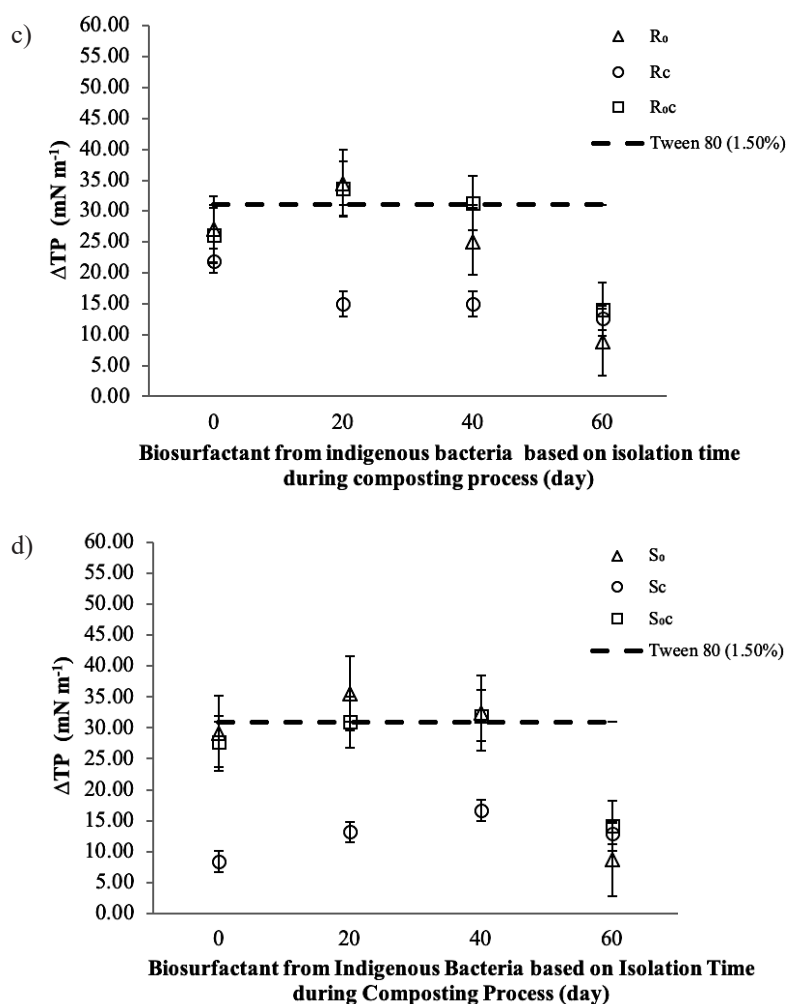


Figure 3c, 3d. Cont. Surface tension decrease of biosurfactants: (a) Mixed crude oil polluted soil with organic waste; (b) Yard waste; (c) Rumen residue; (d) Crude oil polluted soil

52.90 mN m⁻¹, respectively. These variations show different characteristics of biosurfactants depending on the type of media as carbon sources and incubation conditions for bacterial growth (Cameotra and Makkar, 2004; Sakthipriya et al., 2015).

One of the conditions that influence ΔST value of biosurfactant is pH. On the 20th day of S_c, there was a decrease in pH from 6.00 to 4.00 which was also performed at S_c day 0th; S_c day 40th, and S_c day 60th. The decrease in pH value was caused by organic acids and fatty acids from the breakdown of the hydrocarbon from crude oil, which indicated that the decomposition still occurred (Sayara et al., 2010; Zhang et al., 2012). Low pH value causes the biosurfactants to become insoluble, but precipitate instead, which may lead to structure and characteristic changes (Sakthipriya et al., 2015). These changes are caused by fatty acids, which are one of the components of biosurfactant, used by bacteria themselves as a source of

nutrient during the hydrocarbon decomposition process (Chaprão et al., 2015; Trihadiningrum et al., 2018; Zhang et al., 2002). Then, it could be weakened the biosurfactant ability to reduce surface tension. As reported by Zhang et al. (2002) that the ΔST of biosurfactant produced from food waste composting process decreased from 56.92 to 45.13 mN m⁻¹. Meanwhile, the highest ΔST was shown by SW_{oc} day 40th, followed by SW_o day 20th; S_o day 20th; Y_o day 20th; and R_o day 20th of 52.90; 36.90; 35.60; 34.50; and 34.50 mN m⁻¹, respectively. Those biosurfactants are formed at pH 8.00–12.00 that are the preferred conditions for the production of a more stable biosurfactant by bacteria (Raza et al., 2007). Moreover, Hamzah et al. (Hamzah et al., 2013) explained that the highest ΔST could be obtained in the pH range of 8.00–9.00 which corresponds to the SW_{oc} day 40th (see Fig. 2) during the incubation period.

Almost all of the biosurfactants produced in the current experiment was able to reduce the ST value $\geq 10 \text{ mN m}^{-1}$; thus, it can be concluded that it has surface-active properties. In fact, 13 biosurfactants (Fig. 3) were able to reduce surface tension beyond the ability of Tween 80 (1.50%) by 31.00 mN m^{-1} with ratios of 1.01–1.71.

Comparison of emulsification activity between biosurfactant and Tween 80 (1.50%)

The EA formed within 24 hours in all biosurfactants produced ranged from 0.00–12.00% (Fig. 4). The lowest EA, about 0.00%, was found at biosurfactants produced on crude oil media which indicated no emulsion formed. The results are similar to the low EA value of *B. subtilis* strain N2 when grown on hydrocarbon media, as reported by Bezza and

Chirwa (2015). It is influenced by the low concentration of biosurfactants produced by bacteria due to limitation of biodegradable or simpler nutrients, which also affect the bacterial growth (Bezza and Chirwa, 2015; Fatimah, 2007; Hamzah et al., 2013).

Thus, biosurfactant production could be maximized when bacteria are supplied with nutrient-rich media (Bezza and Chirwa, 2015). As reported by Fatimah (2007), the EA of biosurfactant in glucose for a lubricant and hexadecane were 0.03% and 0.02%, respectively. It was followed by greater EA values as reported in the current research, where the highest EA value was found in SW_o and followed by Y_{oc} on day 60th of 12.00% and 10.00%, respectively. These values were close, which indicates that mixed crude oil with organic waste is able to supply the nutrients needed by bacteria. Figure 4 shows that the EA values were lower than Tween 80

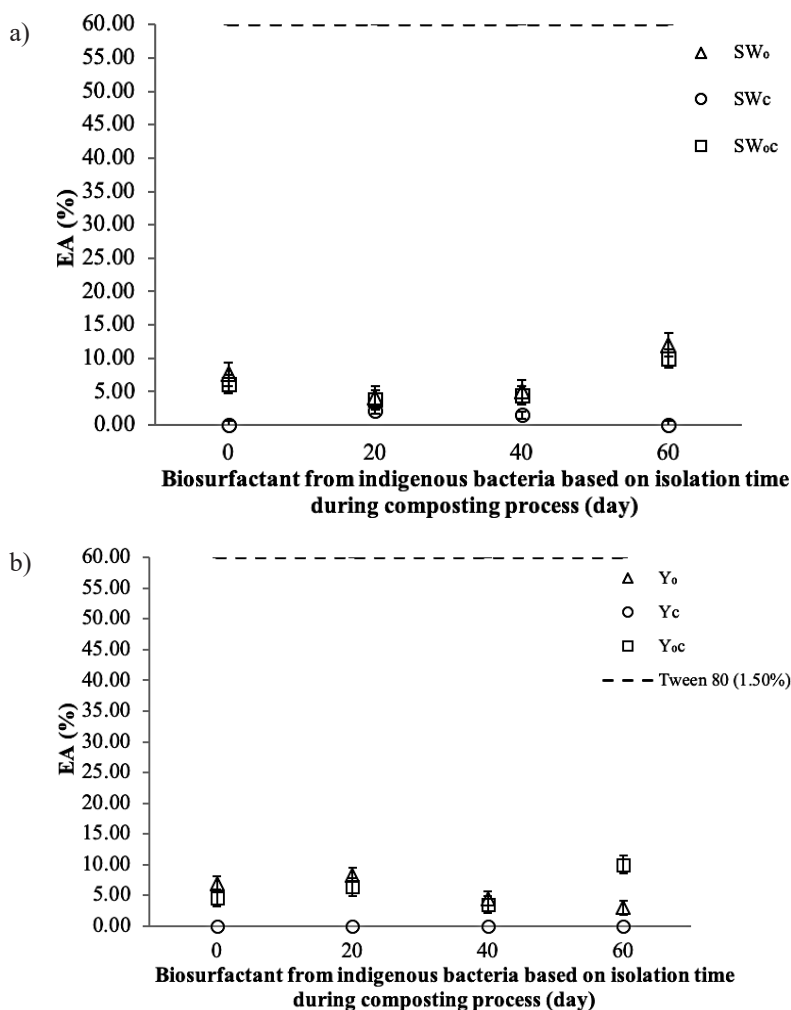


Figure 4. Emulsification activity of biosurfactants: (a) Mixed crude oil polluted soil with organic waste; (b) Yard waste; (c) Rumen residue; (d) Crude oil polluted soil

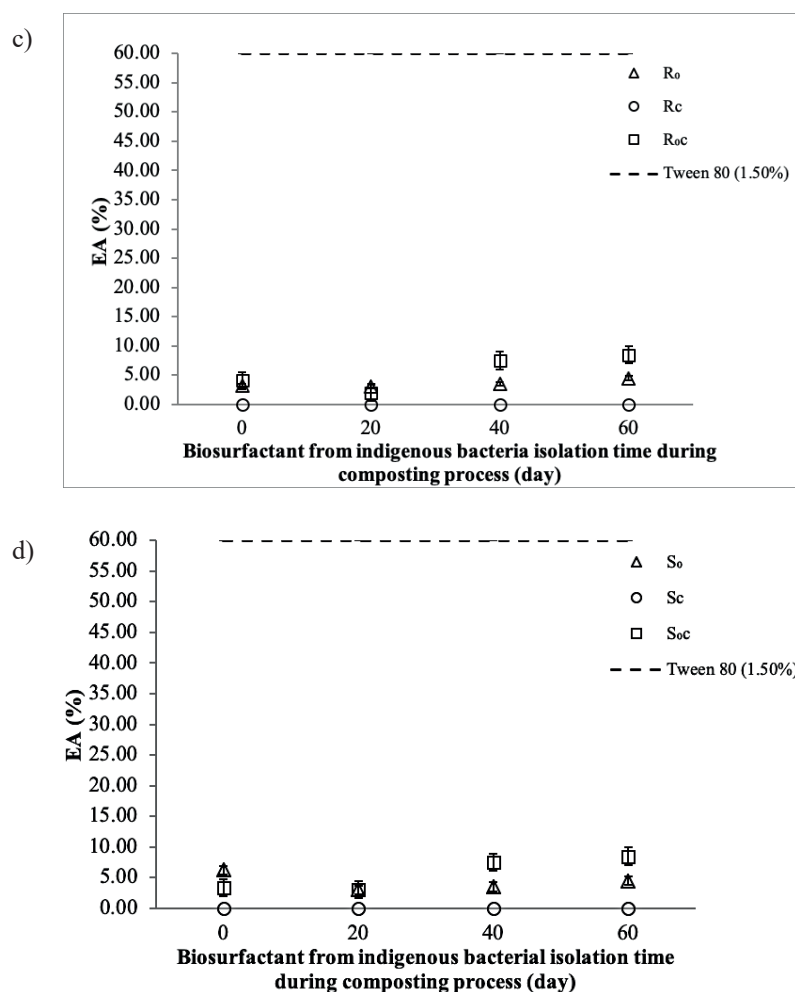


Figure 4c, 4d. Emulsification activity of biosurfactants: (a) Mixed crude oil polluted soil with organic waste; (b) Yard waste; (c) Rumen residue; (d) Crude oil polluted soil

(1.50%) of 60.00% because the synthetic surfactant is more stable. The ratios of the maximum EA value of biosurfactants with Tween 80 (1.50%) were around 0.14–0.20. As a result, the biosurfactants in the current research are able to form emulsions in low activity which had low potential to act as emulsifiers. On the basis of the EA and Δ TTP value, the biosurfactants in the current study are classified as low molecular surfactants (Kazemi, 2017; Pacwa-Płociniczak et al., 2011; Rosenberg et al., 2011) which are known as dispersants (Myers, 2005). The current research has found that the properties of the produced biosurfactant are not only influenced by the number of bacteria, but also by bacterial viability, nutrient bioavailability, and acidity condition formed (Hamzah et al., 2013; Raza et al., 2007; Sakthipriya et al., 2015). This is indicated by the slight difference of indigenous bacteria among all growth media for a 7-day incubation period (Fig. 1). However, in the current

study, no characterization of biosurfactants was carried out considering that the producers were not specific bacteria but a consortium of indigenous bacteria in each composted material. The dominant bacteria found in crude oil polluted soil and its mixture with organic waste, namely *Bacillus sp.* and *B. cereus* which have been reported in our previous study (Sari et al., 2019). The type of biosurfactant is close to lipopeptide (Heryani and Putra, 2017; Zhou et al., 2013) and rhamnolipids (Cerqueira et al., 2011; Tuleva et al., 2005), respectively. This is also supplied by both bacteria which are believed to be hydrocarbonoclastic and lignocellulosic bacteria that act as major producers of biosurfactants due to similarity of hydrocarbon and lignocellulose structures (Bezza and Chirwa, 2015; Zhang et al., 2002). Therefore, sufficient biodegradable organic matter as a source of nutrients has a strong role in stimulating these bacteria to produce biosurfactants simultaneously.

Comparison of hydrocarbon solubilization ability between biosurfactant and Tween 80 (1.50%)

The ability of biosurfactants to solubilize the hydrocarbons from crude oil-polluted soil varied from 841 to 13 620 $\mu\text{g g}^{-1}$ (Fig. 5), where the lowest values was shown by S_c from day 0th to 60th of 841–4684 $\mu\text{g g}^{-1}$. It may be due to the biosurfactants that were unable to form emulsions (EA = 0.00%) and

consisted of a low ΔTP which ranged from 8.35 up to 16.60 mN m^{-1} , as reported in Figure 4. This also occurred in the biosurfactants produced by bacteria from other sources that were carried out on crude oil media (Bezza and Chirwa, 2015). The better hydrocarbon solubilization ability was shown by the biosurfactants produced from mixed organic waste extract with crude oil media, starting from S_{oc} , SW_{oc} , Y_{oc} , and R_{oc} . The S_{oc} produced by indigenous bacteria from composted materials on day

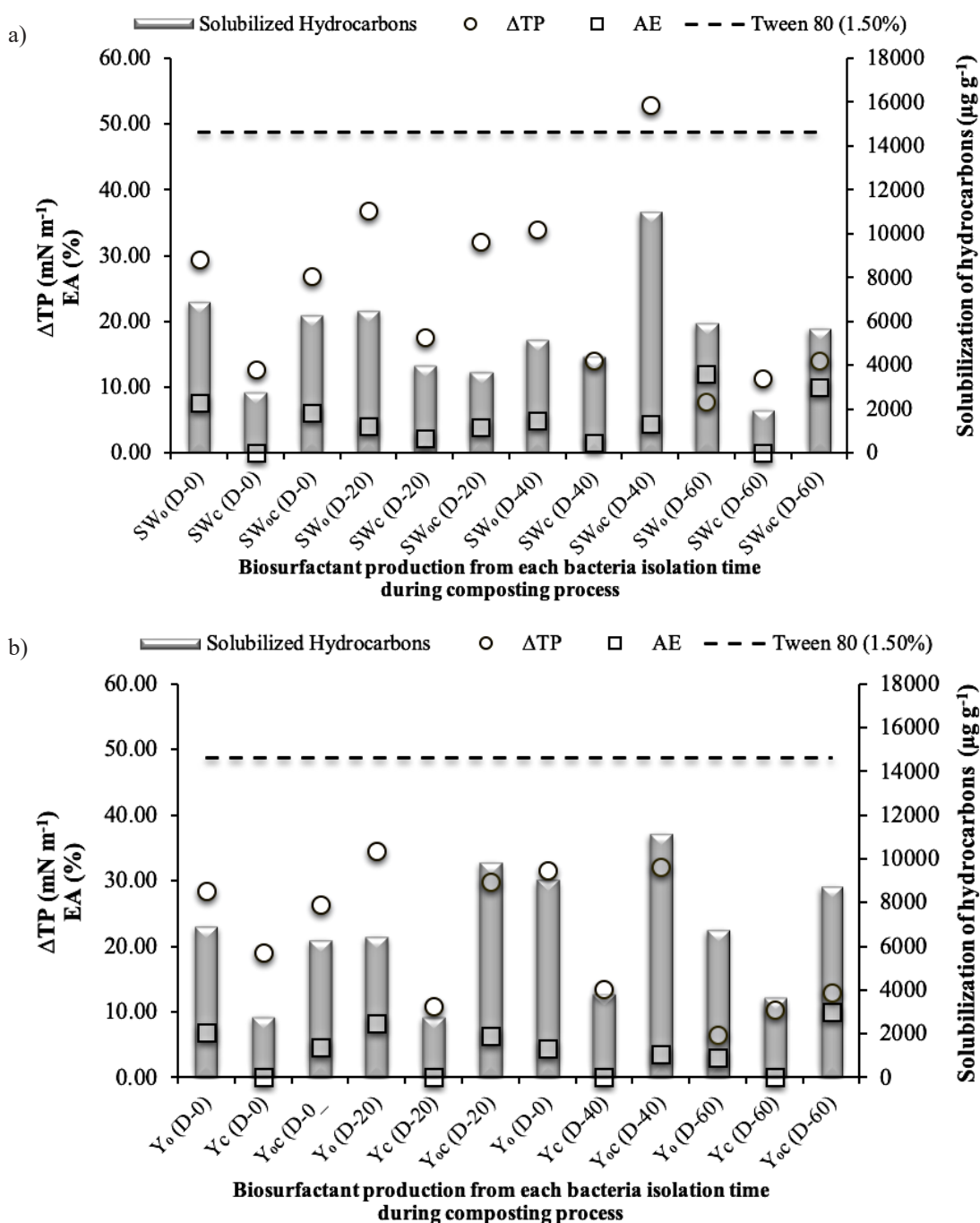


Figure 5. Solubilization of hydrocarbon from crude oil polluted soil by biosurfactants: (a) Mixed crude oil polluted soil with organic waste; (b) Yard waste; (c) Rumen residue; (d) Crude oil polluted soil

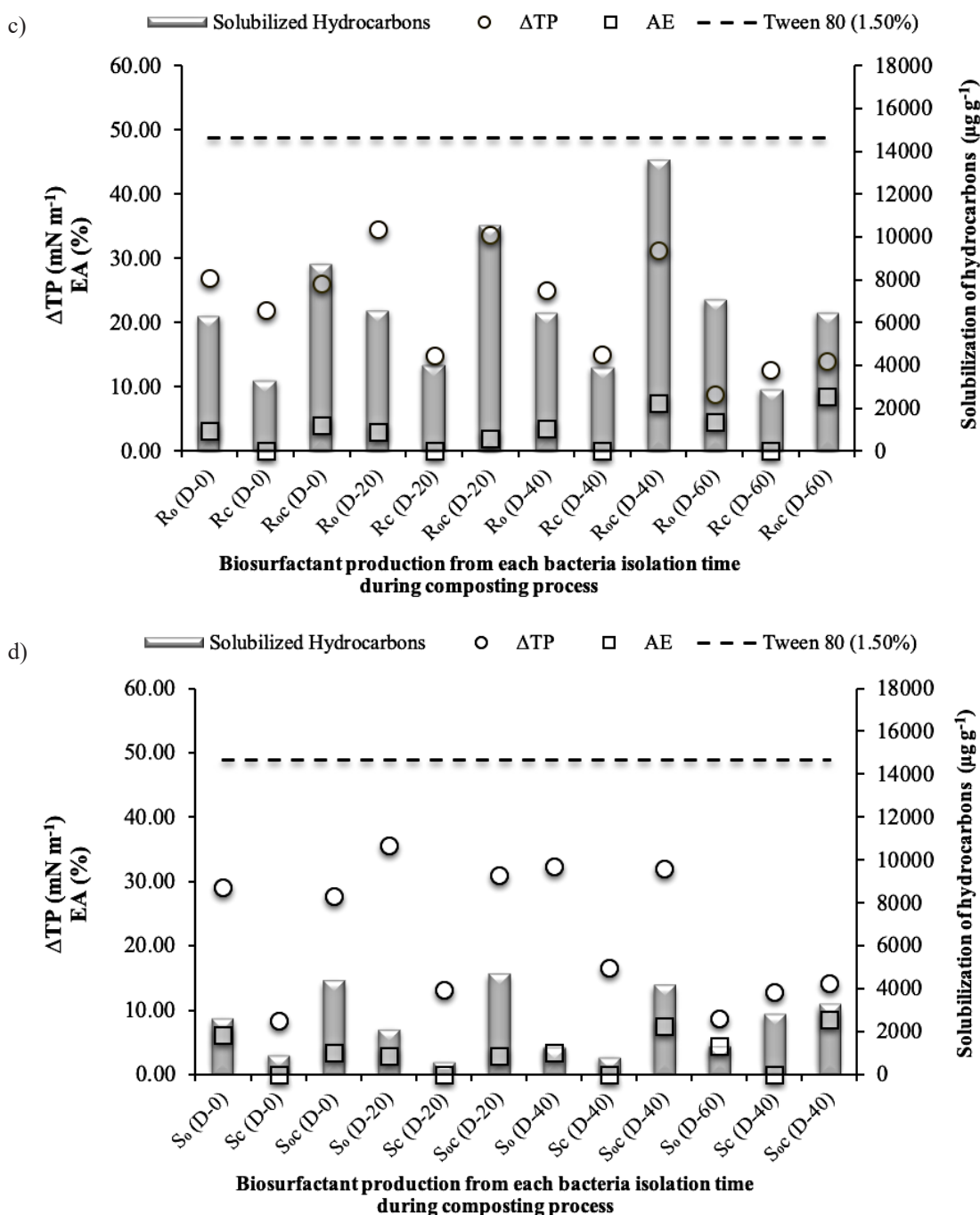


Figure 5 c, 5d. Cont. Solubilization of hydrocarbon from crude oil polluted soil by biosurfactants: (a) Mixed crude oil polluted soil with organic waste; (b) Yard waste; (c) Rumen residue; (d) Crude oil polluted soil

0th, 20th, 40th, and 60th are capable of solubilizing 4384; 4684; 4154; and 3286 μg g⁻¹ of hydrocarbons in crude oil-polluted soil, respectively. In the same period, the biosurfactant produced from SW_{oc} demonstrated a preferable ability to solubilize hydrocarbons of 6300; 3731; 10 998; and 5671 μg g⁻¹, respectively. The SW_{oc} biosurfactant from day 40th showed a higher ability, of 10 998 μg g⁻¹, which was influenced by high ΔTP value of 52.90 mN m⁻¹. Then, greater abilities were also found at day 40th

on Y_{oc} and R_{oc} biosurfactants of 11127 mN m⁻¹ and 13 620 mN m⁻¹, respectively. These results showed that the maximum hydrocarbon solubilization ability was found in the biosurfactants produced from mixed organic waste extract with crude oil media on day 40th, where the values were almost equal to Tween 80 (1.50%) of 14 640 μg g⁻¹. It indicates that the media is able to provide adequate biodegradable nutrients for indigenous bacteria to stimulate the initial metabolic process, then able to adapt to

the hydrocarbon presence. Through the process, potential hydrocarbonoclastics bacteria were triggered by the biosurfactants produced in order to desorb the hydrocarbons from crude oil polluted soil, it was adsorbed and utilized as co-nutrients for further biodegradation process (Kazemi, 2017; Mulligan, 2021). Furthermore, each experiment on day 40th was able to desorb aliphatic hydrocarbons in the C₅₋₃₆ fractions for 4001; 9907; 10 424; and 11 835 µg g⁻¹ of S_{oc}; SW_{oc}; Y_{oc}; and R_{oc}, respectively. Meanwhile, SW_{oc} and R_{oc} biosurfactants on day 40th were also able to desorb aromatic hydrocarbon in the C5-16 fraction of 815 µg g⁻¹ and 1172 µg g⁻¹, respectively. Detailed data for solubilized hydrocarbon fractions were listed in Table 2. Compared to the maximum values of ΔTP and EA (Fig. 5), the ability shown does not illustrate that both of them are the main factors of hydrocarbon solubilization. Singh et al. (2007) explained that ΔTP and EA could have an unpredictable relationship due to widely varied of biosurfactants characteristics. The measured ΔTP and EA were possible not only to stimulate but also to inhibit the remediation process if de-emulsifier characteristics were indicated, which may as well occur in the current study because the EA values were very low. In addition, solubilization hydrocarbons abilities might be influenced by other characteristics of biosurfactant but has not been measured in the current research such as foaming.

Comparison of the hydrocarbon solubilization ability between bio-based surfactant with Tween 80 (1.50%) and its role on crude oil polluted soil composting process

As reported in our previous study, the mixture of crude oil-polluted soil with organic waste (yard waste and rumen residue) at 1:1 ratio (w/w), known as SW, for 150 days of composting process was able to reduce the hydrocarbon level up to 74.68%, which is 31 times higher than polluted soil only (Sari et al., 2019). For the same compost material and process, cHAL (measured in previous experiment) and biosurfactant (current research) are also produced with great hydrocarbon solubilization ability. Therefore, both surface-active capabilities could be combined and considered to act as bio-based surfactant ability (see Table 3) for the composting process.

Table 3 shows that in-situ bio-based surfactants on day 20 and 40 have higher abilities to solubilize hydrocarbon than Tween 80 (1.50%) with the values of 16747; 21619; and 14640 µg g⁻¹, respectively. It turned out that both values were also higher than the ability of Emulgin 1000 (14 220 µg g⁻¹), Tween 20 (13 410 µg g⁻¹), and Tween 60 (12 130 µg g⁻¹) at 1.00% concentration (Torres et al., 2005). Therefore, it can be concluded that in-situ bio-based surfactants which were formed during the composting process of SW have similar abilities

Table 2. Solubilized hydrocarbon fractions during soil washing

Hydrocarbon fractions	Hydrocarbon Concentration during Soil Washing (µg g ⁻¹)											
	S _{oc} , Day-40			SW _{oc} , Day-40			Y _{oc} , Day-40			R _{oc} , Day-40		
	Before soil washing	After soil washing	Solubilized hydrocarbon	Before soil washing	After soil washing	Solubilized hydrocarbon	Before soil washing	After soil washing	Solubilized hydrocarbon	Before soil washing	After soil washing	Solubilized hydrocarbon
Aliph C ₅₋₈	3977	992	2985	6552	1409	5143	3548	1182	2366	12059	3576	8484
Aliph C ₉₋₁₂	123	nd	123	1164	nd	1164	nd	nd	nd	237	nd	237
Aliph C ₁₃₋₁₆	2731	2571	160	2264	271	1993	3417	2530	887	2293	2262	30
Aliph C ₁₇₋₂₀	5248	5111	137	2513	2225	288	9570	6232	3338	7052	5040	2012
Aliph C ₂₁₋₂₄	1635	1245	390	941	nd	941	392	271	121	493	485	8
Aliph C ₂₅₋₂₈	10606	10447	159	6746	6377	369	14460	10994	3466	6763	5962	801
Aliph C ₂₉₋₃₆	2354	2306	47	1567	1559	8	2905	2658	246	4833	4552	281
Arom C ₅₋₈	nd	nd	nd	460	nd	460	nd	nd	nd	419	54	365
Arom C ₉₋₁₂	nd	nd	nd	nd	nd	nd	nd	nd	nd	1137	412	725
Arom C ₁₃₋₁₆	nd	nd	nd	355	nd	355	nd	nd	nd	107	26	82
Arom C ₁₇₋₂₀	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Arom C ₂₁₋₂₄	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Arom C ₂₅₋₂₈	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Arom C ₂₉₋₃₆	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total	26674	22673	4001	22562	11841	10722	34292	23868	10424	35393	22369	13024

Table 3. Comparison of hydrocarbons solubilization ability between bio-based and Tween 80 (1.50%)

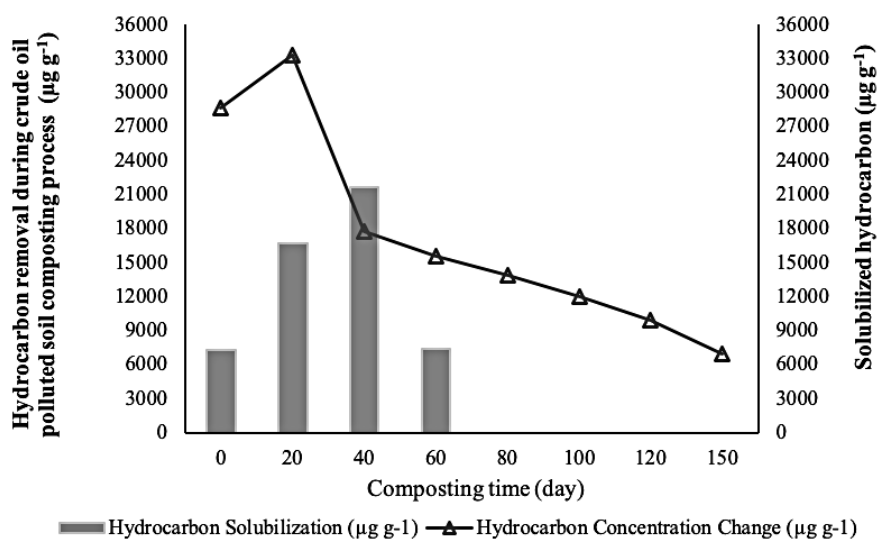
Sample source	Time period	Solubilized hydrocarbon ($\mu\text{g g}^{-1}$)			
		cHAL [10]	Biosurfactant	Bio-based surfactant	Tween 80 (1.50%)
Mixed crude oil polluted soil with organic waste (SW)	0	991	6300	7292	14640
	20	6924	9823	16747	
	40	10621	10998	21619	
	60	1685	5671	7357	

to synthetic surfactants in desorbing hydrocarbons from polluted soil. When observed, the SW component which consisted of yard waste and rumen residue separately have also formed bio-based surfactants that supported hydrocarbon desorption.

The hydrocarbon desorption carried out by in-situ bio-based surfactant of SW is strongly believed to enhance the bioavailability of hydrocarbons for bacteria in the crude oil-polluted soil composting process and is able to optimize degradation, as illustrated in Figure 6. An increase of hydrocarbons concentration was found on day 20, which is thought to be hydrocarbon desorption from the polluted soil matrix. According to Li and Chen (2009) and Mizwar et al. (2016), desorption occurs due to the hydrocarbons from polluted soil matrix that are solubilized in emulsions with in-situ bio-based surfactant. The formed emulsions increase the bioavailability of hydrocarbon for bacteria because it is able to translocate hydrocarbons from the micelles to the hemi-micelles and then to the bacterial cells. It contributes to the utilization of hydrocarbons as a carbon source for indigenous bacteria (Chaprão et al., 2015; Li and Chen, 2009; Zhang et al., 2002). The formation of hydrocarbon

emulsions by in-situ bio-based surfactant during the composting process occurred until the 40th day, which was indicated by an increase in hydrocarbon solubility on day 20 to 40 of $4872 \mu\text{g g}^{-1}$ ($16\,747$ to $21\,619 \mu\text{g g}^{-1}$). The highest solubility and emulsion formed by indigenous bacteria and increased the hydrocarbon degradation performance. It was indicated by the highest reduction of hydrocarbons levels on day 40 of the composting process of $15\,552 \mu\text{g g}^{-1}$. Furthermore, hydrocarbon degradation on day 60 to 150 decreased, which ranged from 1650 – $2924 \mu\text{g g}^{-1}$ (Sari et al., 2019). It may be influenced by the limitation of highly soluble hydrocarbons in the period, as indicated by the decrease of the solubilized hydrocarbon level on day 40 to 60 of $21\,619 \mu\text{g g}^{-1}$ to $7357 \mu\text{g g}^{-1}$, respectively.

The reduction of solubilized hydrocarbon levels was caused by the inhibition of in-situ bio-based surfactant formation due to nutrient depletion of SW during the composting process, indicated by C/N ratio from 34.30 to 17.01 (Sari et al., 2019). In the 40th to 60th day-period of cHAL formation, there was strongly suggested that cHAL had become humin increase (Kulikowska et al., 2015; Sari et al., 2018c). The inhibition has also

**Figure 6.** Solubilization and degradation of hydrocarbons during the crude oil-polluted soil composting process

occurred for biosurfactant, because its production occurred in the log phase of bacteria growth supported by sufficient bioavailable and biodegradable nutrients (Fatimah, 2007; Jiménez-Peñalver et al., 2019; Mulligan, 2021). In order to ensure the stability of in-situ bio-based surfactant formation and high solubility hydrocarbon bioavailability, nutrient adjustment through yard waste and rumen residue addition is required every 40 days. It was expected to encourage bacteria to produce bio-based surfactants simultaneously by utilizing hydrocarbons as co-substrates to increase the effectiveness of hydrocarbon degradation and to accelerate the composting time. Nutrient adjustment before day 40 is not recommended, because it could interfere hydrocarbon degradation, since the bacteria are active in degrading hydrocarbon at days 20 to 40.

In terms of the synthetic surfactant addition or external biosurfactant addition in polluted soil composting process, the effectiveness of hydrocarbon degradation may be improved, by 10.00–23.00% (Chaprão et al., 2015; Li and Chen, 2009; Singh et al., 2007). However, synthetic surfactant addition needs more attention, because it is stable, hard to degrade, and potentially accumulated which has an impact on nutrient and oxygen depletion, and thus can have a toxic effect on microbes in the soil (Fenibo et al., 2019). The addition of synthetic surfactants or external biosurfactant also does not ensure that hydrocarbon is completely and rapidly degraded, because the emulsion formed is not always compatible with bacteria, which inhibits hydrocarbon adsorption into bacterial cells (Aguirre-Noyola et al., 2020; Fenibo et al., 2019). Therefore, the addition of synthetic surfactants or external biosurfactant must be appropriately selected based on its suitability to pollutant characteristics, indigenous bacteria viability, and treatment capacity (Fenibo et al., 2019; Mulligan, 2021). In addition, implementation of synthetic surfactants or external biosurfactant addition require high operational costs and has a potential to increase contaminant exposure (Mulligan, 2021).

Future utilization of bio-based surfactant in crude oil polluted soil composting process

The dynamics of hydrocarbon solubilization and degradation for 60 days showed promising results for periodic addition of lignocellulose waste, especially yard waste and rumen residue (1:1, w/w), for the composting process of crude oil-polluted soil as sustainable bioremediation in the field scale. The

intermittent addition of lignocellulose waste, every 40 days, during the composting process, it can increase the efficiency and accelerate the time of hydrocarbon removal twice. It showed that cHAL and biosurfactant as an in-situ bio-based surfactant that is naturally formed during the composting process of SW can increase crude oil degradation. Therefore, the usage of synthetic surfactants or external biosurfactant as additional supplement on composting process should be replaced by intermittent addition of yard waste and rumen residue. It was also confirmed that the mixture of crude oil-polluted soil with yard waste and rumen residue at 1:1 (w/w) ratio is a suitable composition for sustainable bioremediation through the composting process.

CONCLUSIONS

This work investigated the properties of in-situ biosurfactant production by indigenous bacteria from four composted material for different time-periods, in terms of Δ ST; EA; and hydrocarbon solubilization ability in the range of 8.35–52.90 mN m⁻¹; 0.00–12.00%; 841–13 620 μ g g⁻¹ of C_{5–36} fractions for aliphatic hydrocarbons, respectively. The Δ ST and solubilization ability of SW_{oc} biosurfactant on day 40 of composted material is comparable with Tween 80 (1.50%) in the ratio of 1.71 and 0.75, respectively. Therefore, in-situ biosurfactants are potentially acting as surface tension reducers with low emulsion activity, and classified as low molecular bio-based surfactant.

On the basis of the data from the parts of current research which has been reported earlier, it appeared that the mixture of crude oil polluted soil with yard waste and rumen residue has become a great composition for stimulating biosurfactant and cHAL formation as in-situ bio-based surfactant. In-situ bio-based surfactant at day 40th of composting period has shown higher ability than Tween 80 (1.50%) to solubilize hydrocarbon in the ratio of 1.48. It was confirmed that in-situ bio-based surfactants have an important role as an enhancer of solubility and bioavailability of hydrocarbons for indigenous bacteria to maximize degradation. To ensure the role, the formation of in-situ bio-based surfactants needs to be maintained by adding yard waste and rumen residue periodically, every 40 days of the composting period. The technique could be an appropriate method towards cost-effective sustainable in-situ bioremediation based on a circular waste organic waste management implementation.

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