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Biodegradation of Various Homologues of Palm-based Methyl Ester Sulphonates (MES)

(Biodegradasi Pelbagai Homolog Metil Ester Sulfonat (MES) Berasaskan Sawit)

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ABSTRACT

The concern on the widespread use of surfactants is increasing worldwide as they can be potential toxicants by polluting the environment, with the damage formed depending on their exposure and persistence in the ecosystem. This paper was intended to evaluate the biodegradability of palm-based surfactant, MES, in order to establish their environmental friendliness. The respirometric method was used to monitor the biodegradation of various homologues of MES over 28 days as described in the OECD 301F Manometric respirometry test method. The results showed all the MES homologues tested were readily biodegradable with percentage of biodegradation achieved for C12, C14 and C16 MES was 73% within 6 days, 66% within 8 days and 63% within 16 days, respectively, while linear alkylbenzene sulphonates (LAS) sample 60% biodegraded within 8 days. From the results, it can be concluded that the longer the carbon chain length, the lower is the biodegradability of MES as the microorganisms took longer time to degrade a longer chain surfactant. Other than that, the presence of aromatic structure in LAS may also extend the biodegradation process. The use of palm-based surfactant, i.e. MES, is more environmental friendly and can be used as an alternative to petroleum-based surfactant to reduce adverse environmental effects of surfactant on ecosystem.

Keywords: Environment; LAS; OECD 301F test method; palm-based surfactant

ABSTRAK

Penggunaan surfaktan secara meluas telah meningkatkan kebimbangan pengguna di seluruh dunia kerana bahan ini berpotensi menjadi bahan toksik terhadap alam sekitar, dengan kerosakan yang terbentuk bergantung kepada pendedahan dan kehadiran bahan ini dalam ekosistem. Kajian ini telah dijalankan untuk menilai sifat mesra alam MES berasaskan sawit di sekitaran melalui pengukuran tahap biodegradasi surfaktan ini. Kaedah respirometrik digunakan untuk memantau biodegradasi pelbagai rantaian MES sepanjang 28 hari seperti yang dinyatakan dalam kaedah ujian OECD 301F. Hasil kajian menunjukkan semua homolog MES yang diuji adalah mudah terurai dengan peratusan biodegradasi untuk C12, C14 dan C16 MES adalah masing-masing 73% dalam masa 6 hari, 66% dalam masa 8 hari dan 63% dalam masa 16 hari, manakala sampel linear alkilbenzena sulphonat (LAS) mencapai 60% biodegradasi dalam masa 8 hari. Daripada keputusan tersebut, dapat disimpulkan bahawa semakin panjang rantai karbon MES, semakin rendah peratusan biodegredasi kerana mikroorganisma mengambil masa yang lebih lama untuk menguraikan rantaian panjang ini. Selain itu, kehadiran struktur aromatik dalam LAS juga boleh melambatkan proses biodegradasi. Penggunaan surfaktan berasaskan sawit, iaitu MES, adalah lebih mesra alam sekitar dan merupakan alternatif terhadap surfaktan berasaskan petroleum untuk mengurangkan kesan surfaktan terhadap ekosistem.

Kata kunci: Alam sekitar; kaedah OECD 301F; LAS; surfaktan berasaskan sawit

INTRODUCTION

Surfactants are widely used as active ingredient in many products including household cleaning detergents, personal care products, pharmaceuticals, mining and paper industries. The world production of synthetic surfactants amount is about 13 million tons annually and they are economically important products (Linda 2012). In 2014, the surfactants world market achieved more than 33 billion US-dollars. The market research institute Ceresana, Germany expected the forecasted annual revenues for surfactant will increase by 2.5% p.a. until 2022 (Caresana Research 2015).

Surfactants can be classified into three types, i.e. anionic, nonionic and cationic surfactant. The anionic surfactants are the most commonly used surfactant which is about 60% of the world production, due to their ease and low cost of production. They include alkylbenzene sulfonates (detergents), fatty acid (soaps), lauryl sulfate (foaming agent), di-alkyl sulfosuccinate (wetting agent) and lignosulfonates (dispersants). Most of these anionic surfactants are used for detergent because of their cleaning performance and effectiveness in removing dirt. The nonionic surfactants come next with about 45% of the overall industrial production. These surfactants also have very low sensitivity to water hardness and pH, which makes them very useful in liquid and powder detergents and to stabilize oil-in-water emulsions. Meanwhile, the cationic surfactants were less used because they are more expensive than anionic due to their synthesis process that includes high pressure hydrogenation reaction (Maria 2012; Salager 2002).

The surfactant can be potentially toxicant; therefore when large quantities of surfactants enter the environment via wastewaters, the determination of aquatic toxicity and aquatic behavior of surfactants are of utmost important (Linda 2012). After the detergents have been used, residual surfactants were discharged into the sewage systems or directly into surface waters or river. Other than that, they may also accumulate in huge amount in wastewater treatment plants. Concentrations of discharged surfactants or their degradation products vary in surface waters, sediments and soils amended with sludge (Tomislav & Jasna 2010). For example, the concentration of linear alkylbenzene sulphonate (LAS) in sewage effluent and surface water was reported up to 1.1 and 0.4 mg/L, respectively (Tomislav & Jasna 2010). Due to the nature of surfactants that may pose some adverse affects on human or animal, it is important in future, all new surfactants that will enter the market to have a product safety datasheet, which include the environmental protection parameters such as biodegradability and ecotoxicity.

Methyl ester sulphonates (MES) is an anionic surfactant, which has been successfully used as active ingredient in heavy duty laundry detergents (Zulina et al. 2006) with excellent detergent properties such as good detergency performance, good biodegradability and is less sensitive to water hardness (Masuda et al. 1993; Razmah & Salmiah 2004; Salmiah et al. 1998). This surfactant was produced by converting the fats and oil (including palm oil) to methyl ester, followed by hydrogenation to reduce the unsaturation and then sulphonating the ester to MES. According to Razmah et al. (2006), it was important to evaluate the biodegradability of MES since nowadays, in addition to excellent performance, valuable for money and sustainability; a product must also fulfill the environmental requirements and other regulations in order to be established globally.

Biodegradation is a process whereby microorganisms decompose the organic matter in the environment through utilization of carbon as food. In primary biodegradation, microorganisms may convert the organic molecule into different products, but in ultimate biodegradation they may assimilate the organic molecule completely to a simple molecule (such as carbon dioxide/methane, nitrate/ammonium and water) (Betton 2009). The ability of the chemical compound to degrade is the most significant parameter to determine the adverse effects of the compound on the ecosystem. The percentage of biodegradability of the surfactant must fulfill the standard regulation requirement, for example the European Environmental Safety Legislation such as 73/404/EEC, 82/242/EEC and 73/405/EEC (Uri Zoller 2004) and Regulation (EC) No 648/2004 on detergents.

For reproducible and comparable results between different laboratories, standardized methods have been used globally. There are a number of standardized biodegradability test methods available, such as the ISO standard methods, for example ISO/TR 15462 on water quality-selection of test for biodegradability and the Organisation for Economic Co-operation and Development (OECD) standard methods (OECD 1992). A substance that gave positive result in one of the OECD 'ready biodegradability' tests was assumed to be able to rapidly biodegrade in the aquatic environment, releasing non-toxic products like carbon dioxide and water (ultimate biodegradation). Hence, a substance that was readily biodegradable should not cause any adverse effects to living organisms in the environment (Painters 1992).

This paper was intended to evaluate the biodegradability of various homologues of palm-based surfactant, MES and to compare its biodegradability with petroleum-based surfactant, LAS in order to establish their environmental friendliness.

MATERIALS AND METHODS

Test substances were palm-based MES of various chain lengths (C12, C14 and C16) produced from palm stearin methyl esters in the Malaysian Palm Oil Board (MPOB) MES pilot plant. LAS was purchased from Kong Long Huat, Malaysia and neutralized prior to the biodegradation tests. A reference compound, aniline (99%) from AnalaR-BDH, UK, was used to check the validity of the test procedure.

FOURIER TRANSFORMS INFRARED SPECTROSCOPY (FTIR) ANALYSIS OF SURFACTANT

The FTIR spectra of MES and LAS were recorded using Nicolet Magna-IR 550 spectrometer series II FTIR (Nicolet Instrument Corporations, USA). The sample was scanned between wave numbers 4000 - 500 cm^{-1}, 64 times with a resolution of 6 cm^{-1}.

SOURCE OF INOCULUM

The inoculum used in this biodegradation study was derived from the activated sludge of a wastewater treatment plant treating predominantly domestic sewage. The sludge was collected from Indah Water Konsortium (IWK) Putrajaya, Malaysia and diluted in mineral medium to the concentration as stated in the OECD 301F (1992) test method. Thereafter, the sludge was pre-conditioned by aerating it (in mineral medium) for 5 days at the test temperature (22 ± 2°C).

VIABILITY OF INOCULUM

The viability of bacteria in the inoculum was measured by doing the plate count. All platings were made in triplicate and the plates were then incubated at 37°C. After 24 h of incubation, colonies observed were counted and the number of bacteria in the inoculum was calculated.

PROCEDURE FOR READY BIODEGRADABILITY OECD 301F, MANOMETRIC RESPIROMETRY TEST

All biochemical oxygen demand (BOD) bottles were inoculated with a small volume of the inoculum to give a concentration of 30 mg/L of suspended solids. The mineral medium for the test was prepared as described in OECD 301F (1992). Total volume for each BOD bottle is 300 mL. The oxygen uptakes in all bottles were measured directly using the BOD EVO (Velp, Italy) system for 28 days to produce a BOD curve.

PREPARATION OF BOTTLES

The six BOD bottles used in OECD 301F method were prepared as in Table 1.

TABLE 1. Preparation of BOD bottles

Bottle	Preparation
1 and 2	Sample in mineral medium at 100 mg/L
3 and 4	Reference compound (aniline) in mineral medium at 100 mg/L
5 and 6	Mineral medium only (Blank)

DETERMINATION OF THE THEORETICAL OXYGEN DEMAND (ThOD)

The degree of biodegradation is expressed as a percentage of the ThOD, i.e. the maximum oxygen demand required for complete biodegradation of the test substance. This maximum value is normally calculated from the molecular formula of the test substance. The ThOD for each MES and LAS samples was calculated using the formula described by Gerike (1984).

PERCENTAGE OF BIODEGRADATION

The percentage of biodegradation of the sample was calculated using the following formula.

$$\%\ \text{Biodegradation} = \frac{\text{BOD}\left(\text{mg } O_2/\text{mg test substance}\right)}{\text{ThOD}\left(\text{mg } O_2/\text{mg test substance}\right)} \times 100,$$

where

$$\text{BOD} = \frac{\left[\begin{matrix}(\text{mg } O_2/\text{L uptake by test} \\ \text{and/or reference item})\end{matrix} - \begin{matrix}(\text{mg } O_2/\text{L uptake} \\ \text{by inoculum blank})\end{matrix}\right]}{\text{mg test and/or reference test item/L}}.$$

RESULTS AND DISCUSSION

FTIR ANALYSIS OF SURFACTANT SAMPLES

FTIR spectra can be used as a tool for fingerprint identification of a surfactant. All MES samples (C12, C14 and C16 MES) show the same FTIR spectrum (Figure 1). Figure 2 shows the representative FTIR spectra of MES (C14 MES, blue line) scanned at infrared region of 4000-500 cm^{-1}. The spectrum at 3509 cm^{-1} represents the O-H stretching of carboxylic acid. The presence of ester bond C=O was detected at 1724.6 cm^{-1}, while the peaks between 2955-2851 cm^{-1} correspond to the C-H stretching of alkanes group. Peak at 1052.9 cm^{-1} indicates the presence of sulfonate group due to the S=O stretching vibration (Khaled & Isa 2012). From the FTIR spectrum of C14 MES sample, it can be concluded that this surfactant has a linear structure. As for LAS sample, the FTIR spectrum (red line) shows the presence of C-C aromatic structure at peak 1599 cm^{-1}.

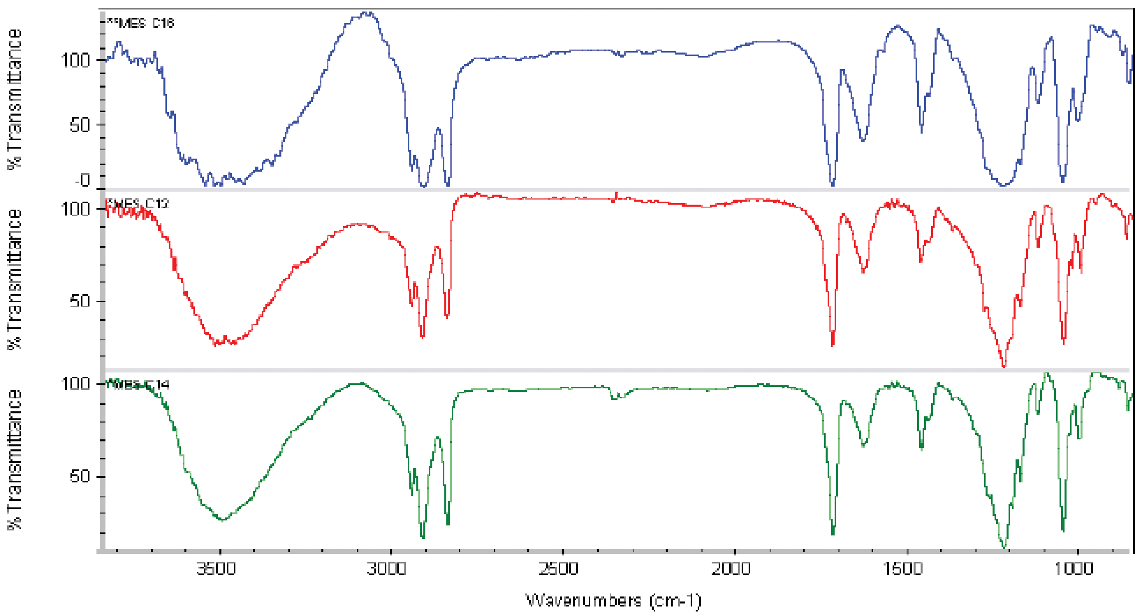

FIGURE 1. FTIR spectrum of C12, C14 and C16 MES

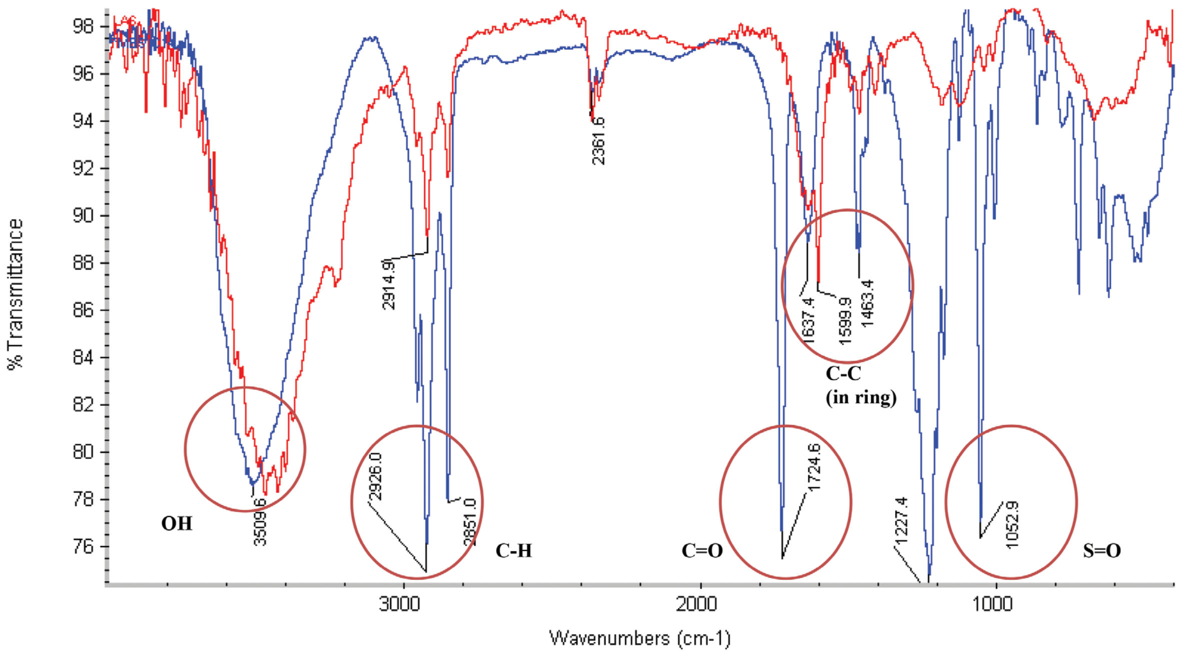

FIGURE 2. FTIR spectrum of C14 MES (blue line) and LAS (red line)

VIABILITY OF INOCULUM

The viability of the collected and pre-conditioned inoculum was measured by performing plate counts for six days. The number of colony count increased from 75 × 10^6 colony forming unit (cfu) at day 0 to 389 × 10^6 cfu at day 6 (Figure 3), showing a 5.2-fold increment of bacteria population. Thus, the pre-conditioned inoculum was well maintained at the test conditions and can be used in the biodegradation test. According to OECD 301F standard method, the concentration of inoculum used in the test should be approximately 10^7 to 10^8cfu.

THEORETICAL OXYGEN DEMAND (ThOD)

The ThOD of MES and LAS was calculated via their elemental composition as described by Gerike (1984). The molecular formula and ThOD of MES and LAS are shown in Table 2. From the table, the ThOD values increase as the chain length of MES increases.

BIODEGRADABILITY OF ANILINE

The biodegradability of the reference compound (aniline) was determined specifically within 14 days from start of test to check the validity of the test and the suitability of the inoculum used. According to the standard method, the biodegradability of aniline should be at least 60% within 14 days. It was observed that its biodegradability reached 100% within 3 days (Figure 4). The validity of this study and the suitability of the inoculum used were thus confirmed.

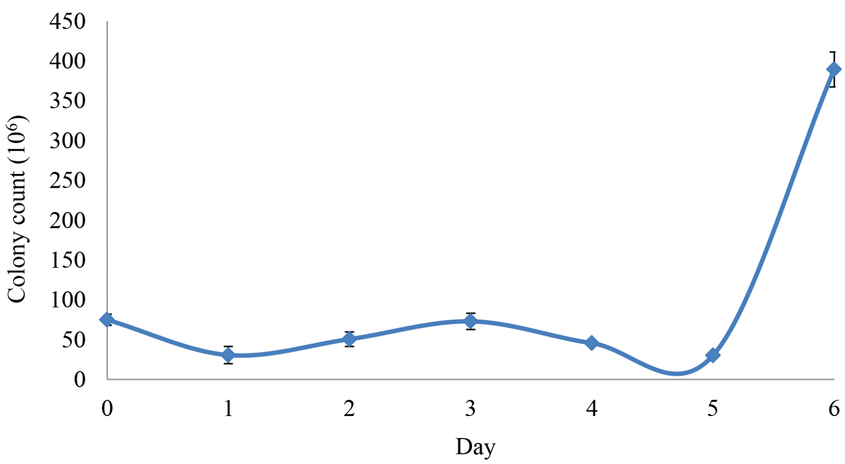

FIGURE 3. Colony counts of inoculum collected from IWK, Putrajaya, Malaysia

TABLE 2. Molecular formula and ThOD of MES, LAS and Aniline

Sample	Molecular formula	Molecular weight	ThOD
C12 MES	$C_{13}H_{25}NaO_5S$	320	1.85
C14 MES	$C_{15}H_{29}NaO_5S$	344	2.00
C16 MES	$C_{17}H_{33}NaO_5S$	372	2.11
C10-13 LAS	$CH_3(CH_2)_{n-1}C_6H_4SO_3Na_{n=10,11,12,13}$	320-362	2.25-2.39
Reference, Aniline	$C_6H_5NH_2$	93	2.41

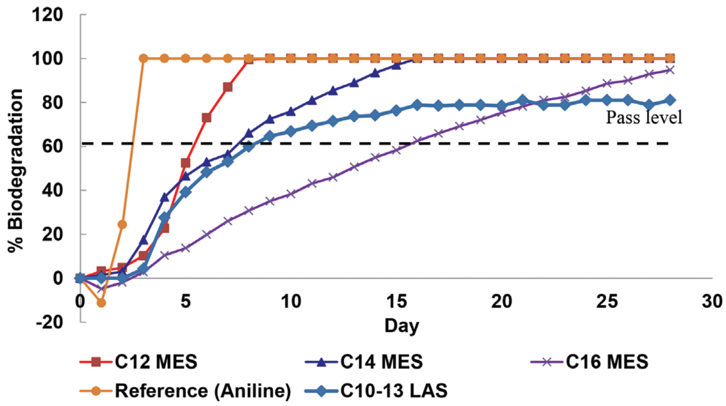

FIGURE 4. Biodegradation curves of MES, LAS (C10-13) and aniline

BIODEGRADABILITY OF SURFACTANTS

The biodegradability of MES (C12, C14 and C16) and LAS (C10-13) determined using method OECD 301F, Manometric respirometry are shown in Figure 4. In principle, any substance meeting the OECD ready biodegradability pass levels (either 60% CO_2 evolution, 60% BOD/ThOD or 70% dissolved organic carbon (DOC) removal within 28 days test period) in one of the biodegradability screening tests are considered as 'readily biodegradable' (OECD 1992). The OECD 301F test method used 60% ThOD as the pass level. The biodegradability of C12, C14 and C16 MES was 73% within 6 days, 66% within 8 days and 63% within 16 days, respectively. From the result, it can be concluded that all MES samples were readily biodegradable because they surpassed the 60% pass level within 28 days and that the longer the carbon chain length of MES, the solubility and the biodegradability rate decreased. In general, biodegradability increases with increasing solubility; solubility is inversely proportional to molecular weight (USGS 2015). In addition, microorganisms may take longer time to degrade any substance with a longer carbon chain length. Based on FTIR spectrum in Figure 2, MES has a linear structure and this made this surfactant easily biodegraded by the microbes compared to other surfactants that were branched or having aromatic moiety. According to Masuda et al. (1994), the biodegradation of MES occurred through ω- and β oxidation, desulfonation (cleavage of C-S linkage) and the oxidation of carbonaceous residues. The biodegradability of MES has also been reported by Razmah and Salmiah (2004). In their report, by using OECD 301D closed bottle test, palm-based MES was found to be readily biodegradable with more than 80% degraded in only eight days. In addition, the MES is not expected to cause environmental concern because its high biodegradability will leave very little residual and therefore is not toxic to aquatic organisms.

The petroleum-based surfactant, LAS, was also readily biodegraded with 60% biodegradability achieved within 8 days (Figure 4). The biodegradability of LAS was lower and slower than the biodegradability of palm-based C12 and C14 MES, although all these three surfactants have about the same carbon chain lengths. LAS has an aromatic moiety based on the FTIR spectrum in Figure 2 and this made the surfactant more difficult to biodegrade.

CONCLUSION

Palm-based MES are readily biodegradable surfactants as shown through the OECD 301F Manometric respirometry standard test method where their biodegradability surpassed the 60% pass level. The results showed that as the carbon chain length of MES increases, it becomes less biodegradable because more time was required by the microbes to degrade this surfactant into simple molecules. Other than that, the presence of aromatic structure in LAS may also extend the biodegradation process. The use of palm-based surfactant, i.e. MES, is more environmental friendly and is the best alternative to replace petroleum-based surfactant and can also help to reduce adverse environmental effects of surfactant on ecosystem.

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