ECOTOXICOLOGICAL BEHAVIOUR OF POORLY WATER SOLUBLE FATTY ALCOHOL ETHOXYLATES IN FRESHWATER ENVIRONMENT

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ABSTRACT
Fatty alcohol ethoxylates (FAEO) are widely used for a wide range of applications. Due to their excessive and widespread use, the ecotoxicological behaviour of FAEO in aquatic environment is crucial in managing the environmental sustainability. The objective of this study is to determine the ecotoxicology behaviour, i.e. biodegradation and ecotoxicity, of poorly water soluble lauryl alcohol ethoxylates (C12) with different ethoxylate numbers, i.e. EO3, EO5, EO6, EO7 and EO10. For ecotoxicity test, OECD 209, activated sludge, respiration inhibition test method was used. This method was selected as a rapid screening test to identify substances that have unfavourable influence on microorganisms in sewage treatment plant and also to identify non-inhibitory concentration of test substances applicable for biodegradation test. Meanwhile, the biodegradation test was performed using OECD 301C, MITI (I) (Ministry of International Trade and Industry, Japan) test method. The biodegradability of this surfactant was monitored for 28 days. The results of OECD 209 showed only FAEO (EO3) with a maximum concentration of 1000 mg litre–1 inhibited more than 50% respiration of activated sludge, while other FAEO samples inhibited less than 50% after 3 hr of exposure. The 3 hr of half maximal effective concentration (EC50) for FAEO (EO3) for activated sludge was 423 mg litre–1 while, for other FAEO samples was > 1000 mg litre–1. The toxicity effect decreased with increasing EO number. For biodegradation test, the results indicated that FAEO were readily biodegraded in OECD 301C where their biodegradability surpassed the 60% pass level as stated in the standard method and can be considered as readily biodegradable in the environment. As the EO chain length increased, the hydrophilic-lipophilic balance (HLB) of FAEO tended to increase while the hydrophobicity tended to decrease. The reduction in hydrophobicity level increases its solubility in water, thus promotes rapid biodegradation in aquatic environment.

Keywords: nonionic surfactant, ecotoxicity, activated sludge, biodegradation.

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INTRODUCTION
Surfactants are surface-active agent that are widely used in household and industrial cleaning detergents, personal care products and pharmaceuticals. The global surfactants market was valued at RM 43 655 million in 2017, and is projected to reach RM 64 408 million by 2025 (Allied Market Research, 2018). Due to their extensive use, surfactants can be a possible toxicant when large quantity enter the environment via wastewaters (Jasna and Tomislav, 2007). Therefore, it is important for all new surfactants to have product safety datasheet, which includes the environmental protection parameters, i.e. biodegradation and ecotoxicity (Linda, 2012).
Some surfactants are poorly soluble in water and they are also discharged with sewage just like the soluble surfactants. This means the biodegradability of poorly water-soluble substances should be assessed and treated the same as soluble surfactants. However, the methodology for measurement of biodegradability of poorly water-soluble substances is less developed compared to soluble substances. According to European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC, 1986), a few standard methods such as closed bottle test, modified Organisation for Economic Co-operation and Development (OECD) screening test, modified Association Francaise de Normalisation (AFNOR) test, modified sturm test and modified Ministry of International Trade and Industry, Japan (MITI) tests are considered suitable for testing poorly water-soluble substances.

Fatty alcohol ethoxylates (FAEO) a nonionic surfactant is widely used in household laundry detergents, industrial cleaners, cosmetics and agriculture (Baumann and Biermann, 1994). FAEO are synthesised via ethoxylation of fatty alcohol (FA) with ethylene oxide (EO). The sources of FA could be either palm kernel oil (PKO), coconut oil or petrochemicals. In 2018 alone, in order to serve high demand from domestic oleochemicals plant, Malaysia increased imports of PKO by 32.4% compared to 2017 (182 106 t) (Kushairi et al., 2019). The solubility of FAEO in water depends on its chain length and the degree of ethoxylation.

The occurrence of FAEO in aquatic environment and sewage treatment plant has been monitored by many countries. In the Netherlands, the concentrations of FAEO in effluent vary between 0.0022 and 0.013 mg litre-1 with an average value of 0.0062 mg litre-1 (Matthijs et al., 1999). The presence of FAEO in the aquatic environment has also been reported in Japanese rivers, where the concentration of FAEO was below the detection limit of 0.005 mg litre-1, whereas the concentration in the sediment ranged from 0 to 1.0 mg kg-1. In Ohio River, USA, the concentration of FAEO C_{14-15} was 0.004 mg litre-1. Meanwhile, total concentrations of FAEO in wastewater treatment plant (WWTP) effluents in Europe, Canada and the United States were between 1 to 23 μ litre-1 (Matthijs et al., 1999; Eadsforth et al., 2006; Morrall et al., 2006). Environmental risk assessments of FAEO have been reported in many studies such as by Goyer et al. (1981), Talmage (1994) and Van De Plassche et al. (1999). These assessments are becoming increasingly sophisticated due to advances in understanding analytical methods, exposure, fate and effects of FAEO in the environment.

Ecotoxicity behaviour of linear FAEO has been observed by Talmage (1994) and HERA (2009) using a few test species such as fish, daphnia and freshwater algae. It was found that the ecotoxicity or half maximal effective concentration (EC_{50}) of FAEO towards algae, daphnia and fish were in the range of 0.1 to 100 mg litre-1. For branched FAEO, toxicity value ranged from 0.5 mg litre-1 to 50 mg litre-1. The EC_{50} values for linear and branched FAEO towards algae were 0.05 mg litre-1 to 50 mg litre-1. The half maximal lethal concentration (LC_{50}) value of linear FAEO towards fish was between 0.4 mg litre-1 and 100 mg litre-1, while for branched FAEO, LC_{50} range was from 0.25 mg litre-1 to 40 mg litre-1. Swisher (1987) suggested the toxicity increases with an increase in the alkyl chain length and decreases with an increase in the ethoxylate chain length.

Relationships between surfactant structure (particularly EO groups) and toxicity towards aquatic organisms have been observed previously. Diana et al. (1997) showed the toxicity of FAEO towards fathead minnow and D. magna tended to increase as the surfactant structure becomes more hydrophobic (high alkyl or low EO chain length). The same observation was made by Maki and Bishop (1979) using D. magna as a test species. According to Hall et al. (1989), ethoxylated alkylphenols (EO>30) showed low toxicity towards Mysisidopsis bahia. The ecotoxicity study by Guhl and Gode (1989) found that the toxicity of three FAEO surfactants (C_{12-18}EO_{10}, C_{16-18}EO_{14} and C_{12-14}EO_{30}) towards fish and Daphnia sp. decreased with increasing EO numbers. Wildish (1976) studied the toxicity effect of surfactants liposolubility towards fish and found that toxicity of polyoxyethylene esters, ethers, and amines in oil dispersants decreased with increasing EO numbers.

The biodegradation of FAEO has been widely studied in many laboratories and field conditions. Most of these biodegradation tests with FAEO were performed under aerobic conditions, and only a few under anaerobic conditions (Markus, 2000). Majority of FAEO biodegradation process occurs in the sewage system or WWTP. Linear alcohol ethoxylate was found to be readily biodegradable and released degradation products such as free fatty alcohol (FFA) and poly(ethylene glycols) (PEG) (Environment Canada, 2003). Kravetz et al. (1984) observed that linear and branched FAEO were biodegraded more than 80% and 40%, respectively, in 28 days.

Although many ecotoxicology studies have been conducted on water-soluble FAEO, limited studies are available for poorly water-soluble FAEO (with EO less than 10). The aim of this work was to study the ecotoxicological behaviour of poorly water-soluble FAEO in order to establish their impact on the environment.
MATERIALS AND METHODS

Materials

The studied test substances were commercial lauryl (C₁₂) alcohol ethoxylates (FAEO) with different ethoxylate numbers, i.e. EO3, EO5, EO6, EO7 and EO10. Table 1 shows the hydrophilic-lipophilic balance (HLB) value and molecular weight of the test substances. Reference substances used for biodegradation test was aniline (99%) from AnalaR-BDH, Germany and 3,5-dichlorophenol (99%) from Sigma Aldrich, USA for ecotoxicity test.

| TABLE 1. HYDROPHILIC-LIPOPHILIC BALANCE (HLB) VALUE AND MOLECULAR WEIGHT OF COMMERCIAL C₁₂ FAEO |
|---------------------------------------------|---------------------------------------------|
| FAEO (EO3)                                  | FAEO (EO5)                                  |
| HLB: 9                                      | 10.5                                       |
| Molecular weight                            | 340                                         |
| FAEO (EO6)                                  | FAEO (EO7)                                  |
| 11                                          | 12                                          |
| 470                                         | 500                                         |
| FAEO (EO10)                                 |                                             |
| 13                                          | 630                                         |

Measurement of Ecotoxicity of FAEO

Sludge inoculum. Activated sludge collected from a sewage treatment plant in Indah Water Konsortium (IWK), Putrajaya, Malaysia, was washed with water. After centrifuging the sludge at 6000 rpm for 10 min, the supernatant was decanted. About 3 g of the washed sludge was dissolved in 1 litre distilled water. The sludge inoculum was aerated for 20 hr before used.

Reference substance. Reference substance 3,5-dichlorophenol was tested at different concentrations, i.e. 1 mg litre⁻¹, 6 mg litre⁻¹ and 12 mg litre⁻¹, under the same test conditions as the test substance in order to check the sensitivity of sludge inoculum.

Preparation of Test Solution

Synthetic sewage feed. The synthetic sewage feed was prepared using analytical grade reagents as given in Table 2. The final volume was 1 litre. The pH of the synthetic feeding solution was set at 7.5 ± 0.5 and stored at 4°C until used.

Six 1 litre schott bottles were prepared for determination of blank, reference substance (3,5-dichlorophenol) and test substance oxygen respiration rate (R) in simultaneous experimental series. Each series was prepared in triplicate. Reference substance was tested at three concentrations, i.e. 1, 6 and 12 mg litre⁻¹, while test substance was tested at 10, 100 and 1000 mg litre⁻¹. In each of test bottle, 16 ml of synthetic sewage feed and 250 ml of sludge inoculum were added. Then, the test bottle was filled with water up to 500 ml. All bottles were closed with a stopper and aerated with air bubbles for 3 hr, then stirred and incubated at 22 ± 2°C in the dark. After 3 hr of incubation, the R value was determined by measuring the decrease of oxygen content for 10 min using dissolved oxygen meter (Multi 3410 WTW, Germany). During the measurement, the stirring was continued but the aeration was stopped.

The percentage of respiration inhibition (Iₜₜ %) was calculated as bellows:

\[ Iₜₜ = 100 - \left( \frac{R_{t} \times 100}{R_c} \right) \]

where,

\[ R_t = O_2 \text{ respiration rate of test item solutions [mg O}_2 \text{ litre}^{-1} \text{ hr}^{-1}] \]

\[ R_c = O_2 \text{ respiration rate of control (mean) [mg O}_2 \text{ litre}^{-1} \text{ hr}^{-1}] \]

| TABLE 2. PREPARATION OF 1 litre OF SYNTHETIC SEWAGE FEED |
|---------------------------------------------------------|
| Chemical                                               | Amount (g) |
| Peptone                                                | 16.0       |
| Meat extract                                           | 11.0       |
| Urea                                                   | 3.0        |
| Sodium chloride (NaCl)                                 | 0.7        |
| Calcium chloride dehydrate (CaCl₂.2H₂O)                | 0.4        |
| Magnesium sulphate heptahydrate (MgSO₄.7H₂O)           | 0.2        |
| Dipotassim hydrogen phosphate (K₂HPO₄)                 | 2.8        |

Measurement of Biodegradation of FAEO

Inoculum. The standard activated sludge (1 litre) was collected at 10 different sampling sites such as sewage treatment plant, industrial waste-water treatment plant, rivers, lakes and seas in the Klang Valley, Malaysia. All the sludge samples from 10 different sampling sites were then mixed together and maintained at 25°C for three months by aerating it in mineral solution (pH 7) containing 0.1% each of glucose, peptone and potassium orthophosphate.

Mineral medium. The mineral medium solutions used in the biodegradation test OECD 301C are described in Table 3.
The concentration of the test substance was 100 mg litre\(^{-1}\) and the inoculum concentration was 30 mg litre\(^{-1}\) dry matter. Other important test parameters for this biodegradation tests are as outlined in the OECD 301C guidelines. The mineral medium solution and test and reference substance stock solutions were prepared using ultra-pure water (Arioso, Human Corporation, Korea).

**OECD 301C, MITI Method**

The biodegradation test was conducted according to OECD 301C MITI test, i.e. coulometer method. In a BOD test bottle, 100 mg of FAEO sample and 30 mg of inoculum were added to 300 ml of mineral medium. The bottles were then stirred for 28 days and the biochemical oxygen demand (BOD) (ppm) was measured continuously with a BOD meter (Coulometer, Ohkura Electric Co. Ltd, Japan). The test substance was prepared in triplicate while reference substance was prepared in duplicate.

**Calculation of Biodegradability**

The biodegradability of FAEO was automatically calculated by the coulometer’s software using the following formula.

\[
\text{BOD} = \frac{\text{mg O}_2 \text{ uptake by test substance} - \text{mg O}_2 \text{ uptake by blank}}{\text{mg test substance in vessel}} = \text{mg O}_2 \text{ per mg test substance}
\]

The concentration of biodegradation was then calculated by dividing the specific BOD with the specific theoretical oxygen demand (ThOD) or chemical oxygen demand (COD).

\[
\% \text{ biodegradation} = \left( \frac{\text{BOD} \ (\text{mg O}_2 \text{ per mg test substance})}{\text{ThOD or COD} \ (\text{mg O}_2 \text{ per mg test substance})} \right) \times 100
\]

ThOD was calculated based on the molecular weight (MW) and chemical formula \((C_cH_hCl_lCl_{1l}N_{n}Na_{a}O_{o}P_{p}S_{s})\).

\[
\text{ThOD (mg mg}^{-1}) = \frac{16[2c + 1/2(h – cl – 3n) + 3s + 5/2 p + 1/2na – o]}{\text{MW}}
\]

Meanwhile, chemical oxygen demand (COD) was used to calculate the percentage of biodegradation when information on the molecular structure of the test substance is not available.

**RESULTS AND DISCUSSION**

Ecotoxicity of FAEO Based on OECD 209, Activated Sludge Respiration Inhibition Test Method

Ecotoxicity of reference substance. The EC\(_{50}\) value of reference sample, 3,5-dichlorophenol was 4.94 mg litre\(^{-1}\) (Figure 1). This value was within the accepted EC\(_{50}\) value for reference substance (2 mg litre\(^{-1}\) to 25 mg litre\(^{-1}\)) as stipulated in OECD 209, activated sludge respiration inhibition test method. Thus, the activated sludge was sensitive and suitable to be used as inoculum in this study.

Ecotoxicity of FAEO. The ecotoxicity of commercial FAEO with different EO numbers (EO3, EO5, EO6, EO7 and EO10) were determined using OECD 209 (2010). The highest concentration used in this study was 1000 mg litre\(^{-1}\). The oxygen respiration rate for all FAEO samples at 1000 mg litre\(^{-1}\) was approximately between 6.42 to 15.18 mg litre\(^{-1}\) hr\(^{-1}\) oxygen (Table 4).
High respiration rate or rapid decrease of oxygen was observed in blanks. This indicates high bacterial activity in the sludge. The inhibition of microbial respiration or decreased oxygen consumption was observed after introduction of FAEO.

At 1000 mg litre\(^{-1}\), only FAEO (EO3) inhibited more than 50%, while other samples less than 50% (Figure 2). FAEO (EO3) had the highest percentage of microbial respiration inhibition, while FAEO (EO10) showed the lowest respiration inhibition percentage. The toxicity level of FAEO decreased as the number of EO increased. The 3 hr-EC\(_{50}\) value for FAEO (EO3) was 423 mg litre\(^{-1}\) (Figure 3). The 3 hr-EC\(_{50}\) for other samples tested were >1000 mg litre\(^{-1}\). According to Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) rating scheme, FAEO (EO3) sample falls under the category of ‘practically non-toxic’ while, others are considered as ‘non-toxic’ (Table 4).

Surfactants may interact with microorganisms, causing inhibition of growth or damage to cellular components. Nonionic surfactants are generally less toxic than cationic and anionic surfactants. Inhibitory activity depends on specific environmental conditions: the types and concentrations of surfactants present, the types and acclimation history of bacterial species, and the presence of foods and other materials. In general, toxicity to bacteria increases with increasing hydrophobic chain length and decreases with increasing ethoxylate chain length (Swisher, 1987).

**Biodegradability of FAEO Based on OECD 301C MITI Method**

**Viability of inoculum.** The viability of the collected and pre-conditioned activated sludge from 10 sites used as inoculum in test method OECD 301C MITI was measured weekly via plate counts for three months. According to the method, the collected inoculum can be used up to four months. The highest number of bacteria was observed after six weeks of aeration (Figure 4), which was 491 fold higher compared to Week 2. After six weeks, the number of bacteria decreased; therefore, the sludge cannot be used as inoculum. According to Nyholm
Figure 2. Percentage of microbial respiration inhibition for FAEO with different EO numbers.

Figure 3. Respiration inhibition (%) of microbial in sample FAEO (EO3).

Figure 4. Colony counts of inoculum.
(1991), due to long pre-culture process in MITI tests, the bacterial diversity in sludge inoculum will decrease and the biodegradation potential may be weakened due to change of bacterial structure.

**Biodegradation of Fatty Alcohol Ethoxylates**

The biodegradability of commercial FAEO with different EO numbers is presented in Figure 5. In the OECD 301 test method, any substance that reaches 60% BOD/ThOD is considered as ‘readily biodegradable’ in the environment (OECD, 1992). Figure 5 shows that all FAEO samples can be considered as ‘readily biodegradable’, where the percentages of biodegradation were more than 60% within the test period. The biodegradation rate of FAEO increased with increasing EO numbers. FAEO (EO10) underwent the highest biodegradability rate, i.e. 76% at Day 28, while the lowest was FAEO (EO3), i.e. 60% in 28 days.

According to Dorn et al. (1993), the biodegradability of alcohol ethoxylate is not affected by the alkyl carbon chain length, but is affected by the molecular structure of hydrophobic chain. Increased solubility though increased EO chain length promotes the biodegradability of alcohol ethoxylates in the environment.

According to Jurado et al. (2013), the biodegradation of ether carboxylic surfactants with shorter alkyl chains was higher than surfactants with longer alkyl chains. For surfactants with similar chain length, biodegradability was higher for those with higher degree of ethoxylation as observed in this study. However, Scharer et al. (1979) and Holt et al. (1992) observed that the biodegradation rate was slow for branched alcohol ethoxylate (EO>20). Nina and Thomas (2004) showed that alcohol ethoxylate was readily biodegradable in activated sludge but the biodegradation rate varied depending to its carbon chain length and number of EO.

The biodegradability of nonionic surfactants is simultaneously influenced by several parameters such as hydrophobic structure, linearity of the carbon backbone, length of the alkyl chain, the type of bond to the EO, and its length. According

![Figure 5. Biodegradation curves of FAEO samples.](image-url)

![Figure 6. The correlation between FAEO biodegradation and HLB values.](image-url)
to Jurado et al. (2007), there seems to be a positive correlation between biodegradation and the HLB values. HLB describes the relationship between water-soluble and oil-soluble parts of nonionic surfactant. Higher HLB value indicates higher solubility of the surfactant in water.

The results in this study showed that by increasing the EO number or the polar head of FAEO, the HLB value tended to increase while the hydrophobicity decreased. The reduction in hydrophobicity level of FAEO increases its solubility in water, thus promotes rapid biodegradation in aquatic environment (Figure 6).

CONCLUSION

The results of OECD 209, activated sludge, respiration inhibition test, showed FAEO (EO3) sample with a maximum concentration of 1000 mg litre⁻¹ inhibited more than 50% respiration of activated sludge, while other samples did not cause more than 50% respiration inhibition to activated sludge after 3 hr of exposure. All FAEO samples can be considered as practically non-toxic to non-toxic towards microbial respiration. The toxicity effect of FAEO decreases with increasing ethoxylate number. Evaluation of biodegradability of poorly water soluble substances can be done using ready biodegradability test, i.e. OECD 301C MITI. The FAEO samples were readily biodegraded using this test method where their biodegradability surpassed the 60% pass level and can be considered as readily biodegradable in the environment as stated in the standard method.

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