EVALUATION OF ECOTOXICITY OF TYPICAL SURFACTANTS FOR LEATHER MANUFACTURE BY LUMINESCENT BACTERIA

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Abstract. Surfactants are used as auxiliaries in every wet processing process of leather production and discharged into wastewater, which would cause potential ecological risks. In this paper, fresh luminescent liquids were employed to evaluate the ecological toxicity of six surfactants, including anionic, cationic and non-ionic surfactants, and mixture of two typical ionic and nonionic surfactants after a 15-min exposure period. Non-ionic surfactants AEO and Tween80 showed slight light inhibition ie.10-35% to luminescent bacteria. The toxicity of anionic surfactants with polar sulfonic group was: penetrant T(EC_{50}=406.81mg/L) > SDBS(EC_{50}=573.37mg/L). The toxicity of cationic surfactants was: DTAB (EC_{50}=10.68mg/L) > SKC (EC_{50}=73.96mg/L). The addition of nonionic surfactants reduced the toxicity of ionic surfactants. 1-1 mixture of SKC and AEO: EC_{50}=80.17mg/L, 1-1 mixture of SDBS and AEO: EC_{50}=624.34mg/L. These results provided ecological parameters for the selection of surfactants in the process of ecological leather production.

1 Introduction

The process of leather production is heterogeneous physical chemical reaction. Therefore, the interfacial property has an effect on the leather processing and the product performances[1]. Surfactants involving in interfacial properties were extensively used in the wet processing process of leather production to improve the permeation, diffusion, absorption or spreading of other leather chemicals[2-4]. According to the features of dissociation activity of polar groups, surfactants are divided in to ionics and nonionics, in which the ionic surfactants are divided into anionics, cationics, zwitterionics. Anionic and nonionic surfactants are in widespread application in leather production, such as soaking, degreasing, liming, tanning, dying, and fatliquoring[5-6]. Meanwhile, cationic surfactants have been increasingly applied in leather production, due to the special properties of softening and sterilization[7]. Besides, surfactants compound are extensively used in leather production due to the excellent properties, such as strong surface/interfacial activities, low cost, low critical concentration (cmc), ect, compared to the single surfactant[8-10].

It is generally viewed that the surfactants are non-covalently bonded to collagenous fibers in leather processing, resulting in that surfactants are discharged in to the tannery wastewater[11-12]. It is widely acknowledged that surfactants will reduce the content of dissolved oxygen in water. For instance, anionic surfactant sodium dodecyl benzene sulfonate (SDBS, the COD value is up to 13g/L) has significantly effects on oxygen restoration process and physical chemical reactions of other organics in water body, which has potential ecological risks on the ecosystem[13]. Toxicity testings with several biological systems are used to evaluate aquatic toxicity, such as *algae*, *fish*, *Daphnia magna*, and luminous bacteria[14]. The bioluminescent bacteria assay is adopted in worldside countries as standard method for its short test time and high sensitivity[15-16].

In this paper, bioluminescence inhibition assay with luminous bacteria (*Photobacterium Phosphoreum*) was introduced to evaluate the ecotoxicity of nine common anionic, cationic, nonionic and mixed surfactants.

2 Materials and Methods

2.1 Test Chemicals

Stock solutions of anionic surfactants penetrant T (AR, Chengdu, China) and Sodium alkyl benzene sulfonate (AR, Chengdu, SDBS), cationic surfactants dodecyl trimethyl ammonium bromide (DTAB, AR, Chengdu, China) and benzyl dimethyl stearyl ammonium chloride (SKC, AR, Chengdu, China), nonionic surfactants fatty alcohol polyoxyethylene ether (AEO, CP, Chengdu, China) and Tween 80 (AR, Chengdu, China) were prepared in saline water solution (3% sodium chloride [NaCl]) in all of experiments. SDBS and AEO were mixed at different mass ratio 1:1, and the same to SKC and AEO. The mixed surfactants were also prepared in 3% NaCl. Water used in all the experiments was distilled.

2.2 Bacterial Culture

The freeze-dried *Photobacterium phosphpreum* (T3 mutation) was purchased from Shenzhen Langshi Biological Instrument Co., Ltd. (Shenzheng, China). The reagent was stored at -20 °C and rehydrated before inoculation. The bacteria were cultured in the complete liquid culture medium (5.00g tryptone, 5.00g yeast extract, 30.00g NaCl, 12.61g $Na_2HPO_4 \cdot 12H_2O$, 1.31g $K_2HPO_4 \cdot 3H_2O$, 3.00g glycerin, and 1000mL distilled water) with a shaking speed 200rpm at 20°C for 18h. Then the pre-culture *P. phosphereum* was inoculated in new complete liquid medium again. And the bacteria were grown to the logarithmic growth stage after 18h[17].

2.3 The Determination of Bacterial Density for Test

5mL fresh bacteria liquid in the logarithmic growth stage was diluted with 11mL 12mL 13mL 14mL 15mL 16mL 17mL 18mL 19mL 20mL 3% NaCl to obtain bacteria suspensions in different optical density (OD) which can be determined at 600nm with UV-vis spectrophotometer (Shanghai MAPADA Co. Ltd., China). 50μL bacteria suspensions in different optical density were added to the 950μL 0.10mg/L $HgCl_2$ (as standard controls) and 3% NaCl (as blanks). After an exposure for 15min at 20°C, the relative luminous intensity was detected by LumiFox 6000 (Shenzhen Langshi Biological Instrument Co., Ltd., China). All samples were tested in three parallel samples. The 50±5% bioluminescence inhibition of 50μL bacteria suspension served as a reference to verify the reliability of the experimental results.

2.4 Toxicity Test

According to the National Standard Method of China (GB/T 15441-1995, 1995), 50μL bacteria suspensions were added to 950μL test chemicals and the control (3% NaCl solution), respectively. At least 10 concentrations were prepared for every surfactant. The relative luminous intensities of the bacteria with an exposure of 15min at 20℃ were measured. All samples were tested in triplicate.

2.5 Data Analysis

The relative luminous intensity E of sample could be calculated as follows:

$$E = \frac{I}{I_0} \times 100\%$$

where I_0 and I were average luminous intensity of *P. phosphoreum* exposed to the blank controls and test samples, respectively. The regression models were used to describe dose-effect relationships of the surfactants on the bacteria. Toxicity of the samples was evaluated by EC_{50}, the effective concentration corresponding to 50% bioluminescence inhibition calculated from the models. The higher EC_{50} value indicated lower toxicity[17-20].

3 Results and Discussion

3.1 Bacteria Density and Sensitivity Measurement

The relationship between OD_{600} value and the relative luminous intensity of the bacteria suspension after exposed to 0.10mg/L $HgCl_2$ was shown in Fig. 1

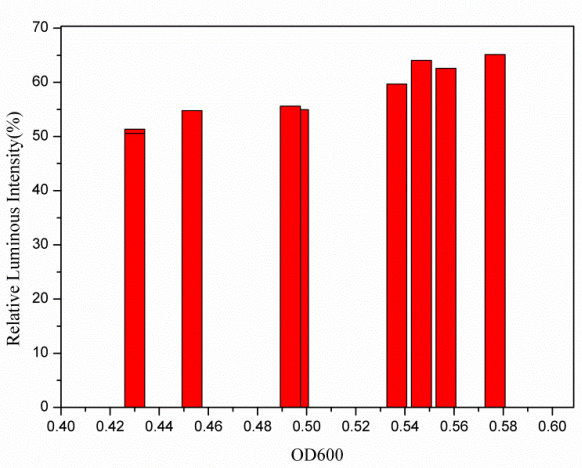

Fig. 1. The relationship between OD_{600} and relative luminescence intensity of bacterial liquid.

Obviously, the density of the bacteria suspension affected the relative luminous intensity, thereby affecting the sensitivity and accuracy. As shown in Fig. 1, the relative luminous intensity of bacteria increased with the increase of bacteria density after the exposure of 0.10mg/L $HgCl_2$. There was no clear linear relationship between them, which may be one of sources of the errors. However, when the bacteria density within certain range, e.g. OD600 in 0.55~0.57, the relative luminous intensity of bacteria exposed to 0.10mg/L $HgCl_2$ fall in 50±5%, which meant that the results has excellent stability. Therefore, it is necessary to confirm the bacteria density by standard toxic substance (0.10mg/L $HgCl_2$) when the fresh bacteria liquid is used to detect the toxicity of chemicals.

3.2 Toxicity of Nonionic Surfactants

AEO and Tween 80 are polyethoxylated nonionic surfactants, which are widely applied in soaking, degreasing and pickling et al. in leather production. The dose-effect relationships of nonionic surfactants were displayed in Fig. 2.

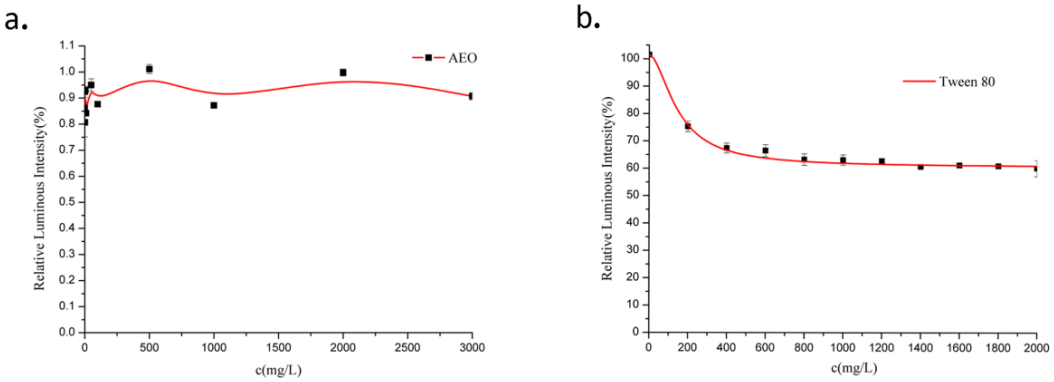

Fig.2. Dose-effect relationship of anionic surfactants on luminescent bacteria, a: AEO b: Tween 80.

As stated in Fig. 2, the inhibitory effects of the surfactants to *P. phosphoreum* were limited in the range from 0.1mg/L~2000mg/L. And there are differences among them in the laws of inhibitions. The relative luminous intensity always fluctuated around 85% with AEO concentration from 0.1mg/L to 3.0 g/L, which indicated little ecotoxicity on the bacteria. The relative luminous intensity of bacteria exposed to Tween 80 decreased to 68% with the concentration increasing from 1mg/L to 400mg/L and remained invariable as the concentration increased to 2.0 g/L. The EC_{50} of the surfactants couldn't be obtained in the range of the testing concentration. Based on Microtox toxicity grading standard of America (the relative luminous intensity < 25% for very toxicity, 25~50% for moderately toxicity, 51-75% for toxicity, 75% for slightly toxicity), AEO and Tween 80 (<0.2g/L) showed slightly toxicity for *P. phosphoreum*.

3.3 Toxicity of Anionic Surfactants

SDBS (Sodium alkyl benzene sulfonate) and penetrant T (sulfonated alphatic polyester) are sulfonate anionic surfactant and used in soaking, liming, dying, fat liquoring and so on. The dose-effect relationships of the two surfactants were stated in Fig. 3.

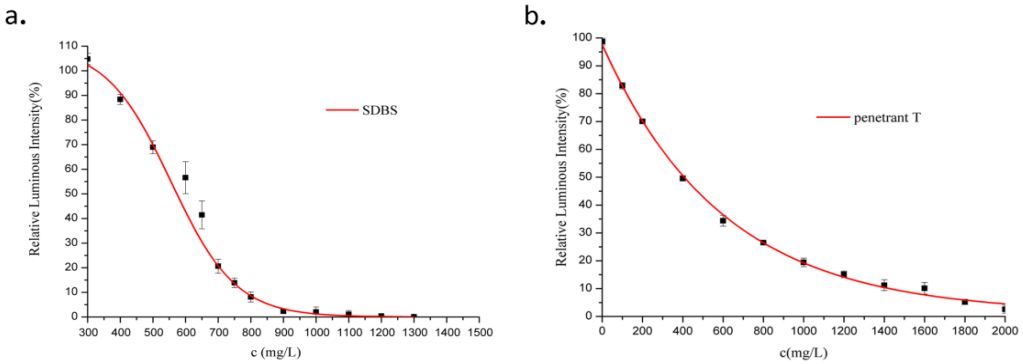

Fig.3. Dose-effect relationship of anionic surfactants on luminescent bacteria, a: SDBS; b: Penetrant T.

As displayed in Fig. 3, the bioluminescence inhibition values increased and the relative luminous intensities decreased with the increase of the concentration of the surfactants. When the mass concentration of SDBS increased to 1100 mg/L, the bioluminescence was inhibited completely and the relative luminous intensities decreased to 0%. The model DoseResp wasd used to fit the dose-effect curve of SDBS as follows:

$$y = 2.7834\times10^{-4} + \frac{1.1009 - 2.7834\times10^{-4}}{1+10^{(555.0962-x)\times(-0.0044)}}.$$

The correlation coefficient of the model was 0.9986 and EC_{50} was 598.15mg/L. While, as the mass concentration of penetrant T increased to 2000mg/L, the bioluminescence was also inhibited and the relative luminous intensities of the bacteria decreased to 5%. The dose-effect relationship of penetrant T was described by the regression model ExpDec1 as follows:

$$y = 0.9649e^{-\frac{x}{598.3981}} + 0.0111.$$

The correlation coefficient of the model was 0.9995 and EC_{50} was 406.81mg/L. According to the bioluminescence inhibition of the bacteria, penetrant T showed higher toxicity than SDBS. Based on the American Microtox toxicity grading standard, the relative luminous intensities was lower than 50% when the concentration of SDBS and penetrant T exceeded 200mg/L, indicating that the two surfactants showed moderately toxicity on the bacteria. However, on the basis of the toxicity standard of BASF SE, the chemicals of which EC_{50} are greater than 100mg/kg are considered to be safe. So, SDBS and penetrant T are safe products.

3.4 Toxicity of Cationic Surfactants

SKC (benzyl dimethyl stearyl ammonium chloride) and DTAB (dodecyl trimethyl ammonium bromide) are quaternary ammonium cationic surfactants, applied in the soaking, pickling, tanning, fatliquoring, finishing et al. The dose-effect relationships of the two surfactants were shown in Fig. 4.

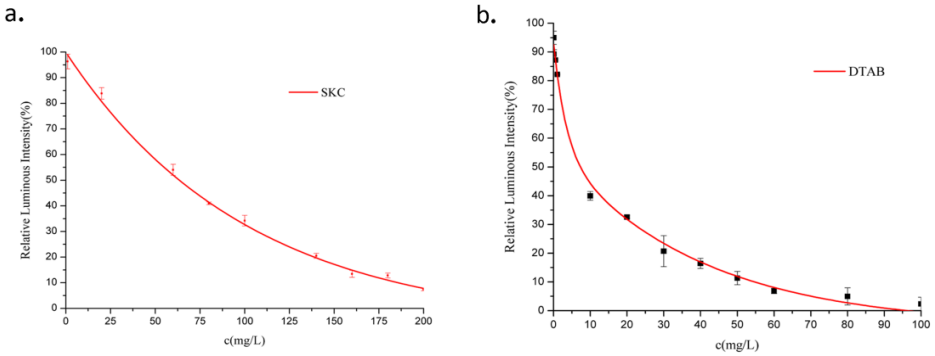

Fig.4. Dose-effect relationship of cationic surfactants on luminescent bacteria, a: SKC; b: DTAB.

As shown in Fig. 4, with the concentration of the three surfactants increasing, the bioluminescence inhibition increased significantly and the relative luminous intensities decreased rapidly. The relative luminous intensity deceased to 10%, when the concentration of SKC increased to 200mg/L. The dose-effect relationship of SKC was fitted by the model ExpDec3:

$$y = 66.6813e^{\frac{-x}{206.5416}} + 43.3805e^{\frac{-x}{206.4838}} + 30.8815e^{\frac{-x}{206.7109}} - 48.5245 .$$

The correlation coefficient of the model was 0.9930 and EC_{50} was 73.96mg/L. When the concentration of DTAB increased to 20mg/L, the decreasing trend of the relative luminous intensity became slower. And when the concentration increased to 120mg/L, the bioluminescence was inhibited almost completely. The model ExpAssoc was used to describe the dose-effect relationship of DTAB as follows:

$$y = 0.9540 - 0.5052(1 - e^{\frac{-x}{35.7272}}) - 0.4441(1 - e^{\frac{-x}{8.1927}}) .$$

The correlation coefficient of the model was 0.9995 and EC_{50} was 10.68mg/L. The values of EC_{50} indicated that DTAB are more toxic than SKC. According to the toxicity standard of BASF SE, the chemicals of which EC_{50}s are greater than 100mg/kg are considered to be safe. It is meant that the surfactants in the range from 1~100mg/L can be used safely.

3.5 Toxicity of the Mixture of Surfactants

The toxicity of mixture of ionic surfactants (including anionics SDBS and cationics DTAB) and nonionic surfactant (AEO) was detected and the dose-effect relationships was shown in Fig. 5.

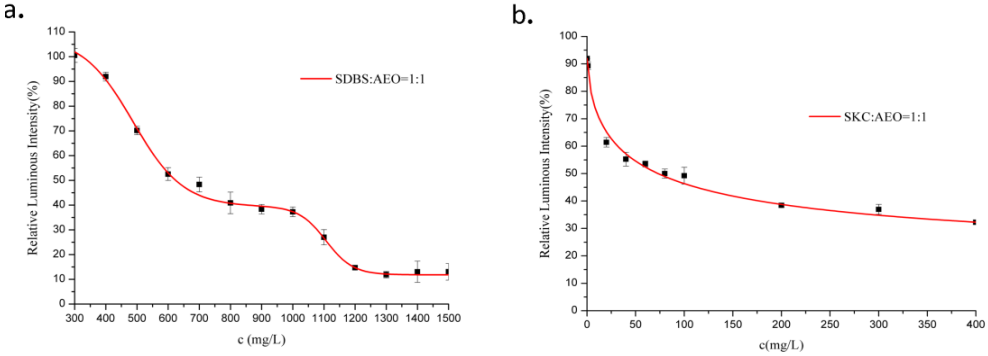

Fig. 5. Dose-effect relationship of the compound of surfactants on luminescent bacteria, a: mixed surfactants of SDBS and AEO; b: mixed surfactants of SKC and AEO.

As shown in Fig.5, the relative luminous Intensity decreased with the increase of concentration of the surfactants mixtures. In consideration of little toxicity of AEO, only concentrations of individual ionic surfactants were stated as x-axis in the dose-effect relationships of the mixed surfactants. The dose-effects relationship of the mixture of SDBS and AEO was fitted by BioDoseResp:

$$y = 0.1183 + (1.0811 - 0.1183)\left[\frac{0.7138}{1+10^{(487.2145-x)\times(-0.0054)}} + \frac{1-0.7138}{1+10^{(1106.4488-x)\times(-0.0100)}}\right].$$

The correlation coefficient of the model was 0.9994. The EC_{50} was 624.34mg/L, lower than EC_{50} of individual SDBS 598.15mg/L indicating that the composites of SDBS and AEO resulted in the lower toxicity of the surfactants. For the mixture of SKC and AEO, the relative luminous intensity decreased with the increase of the surfactant mixtures and maintained about 35% with the concentration increasing to 400mg/L. The dose-effect relationship was described by the model Logistic:

$$y = 14.1394 + \frac{93.7710 - 14.1394}{1 + (\frac{x}{52.5623})^{0.6010}}.$$

The correlation coefficient of the model was 0.9998. The EC_{50} was 80.17mg/L. Though the EC_{50} of the mixtures was closed to the EC_{50} of individual SKC (73.96mg/L), the relative luminous intensity remained about 35% when the concentration exceeded 100mg/L, which meant that the individual SKC showed much higher toxicity than the mixtures of SKC and AEO. To sum up, the mixture of ionic and nonionic surfactants showed lower toxicity than individual ionic surfactants.

4 Conclusion

In this work, the ecotoxicity of the surfactants widely used in leather production were investigated using bioluminescence inhibition assay with *Photobacterium phosphoreum*. The cationic surfactants showed highest toxicity on the bacteria and the nonionic surfactants showed lowest toxicity. And the composites of ionic and nonionic surfactants could lower the toxicity of surfactants.

The testing results provide the evaluation parameters of the eco-friendliness for the selection of the surfactants in leather production. Meanwhile, the purity of the industrial chemicals can't meet the requirement of the experiment reagents. The coexisting chemicals have complicate impacts on the toxicity of the surfactants. Therefore, it is necessary to investigate the toxicity of the surfactants in practical application.

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