Biodegradation (2007) 18:283–293
DOI 10.1007/s10532-006-9062-x

ORIGINAL PAPER

# Effect of surfactants on plasticizer biodegradation by *Bacillus subtilis* ATCC 6633

**Adam R. Grochowalski · David G. Cooper · Jim A. Nicell**

Received: 7 December 2005 / Accepted: 2 June 2006 / Published online: 2 November 2006


**Abstract** The biodegradation of plasticizers has been previously shown to result in the accumulation of metabolites that are more toxic than the initial compound. The present work shows that the pattern of degradation of di-2-ethylhexyl adipate by *Bacillus subtilis* can be significantly altered by the presence of biosurfactants, such as surfactin, or synthetic surfactants, such as Pluronic L122. In particular, this work confirms that the monoester, mono-2-ethylhexyl adipate, is a metabolite in the breakdown of the plasticizer. This metabolite was proposed but not observed in earlier studies. Toxicity measurements showed it to be significantly more toxic than the plasticizer. Thus, the effect of the surfactants was to significantly increase the accumulation of one or both of the two most toxic metabolites; i.e., the monoester and 2-ethylhexanol. It was proposed that the most likely cause of the effect of the surfactants was the sequestering of these two metabolites into mixed micelles, resulting in their reduced availability for further degradation.

A. R. Grochowalski · D. G. Cooper (✉)
Department of Chemical Engineering, McGill University, 3610 University, H3A 2B2 Montreal, QC, Canada
e-mail: david.cooper@mcgill.ca

J. A. Nicell
Department of Civil Engineering and Applied Mechanics, McGill University, 817 Sherbrooke Street West, H3A 2K6 Montreal, QC, Canada

**Keywords** Biodegradation · DEHA · 2-Ethylhexanol · Mono-2-ethylhexyl adipate · Plasticizer · Surfactant

## Introduction

A consequence of the growth of the plastics industry is a significant increase in the amount of plasticizers released into the environment (Cadogan et al. 1999; Peakall et al. 1975). These lipophillic compounds are added to plastics in order to impart a variety of desired properties to the formulations (Wilson 1995). They are not covalently bound to the plastics in these mixtures and are susceptible to leaching (Bauer and Herrman 1997; Fujii et al. 2003; Hill et al. 2003). Since plastic formulations, such as those used with PVC, can contain up to 40% plasticizer by weight (Graham 1973), even a very gradual loss of plasticizer can become a very significant source of environmental contamination. Consequently, these compounds have become ubiquitous in the environment (Staples et al. 1997; Fromme et al. 2002; Kolpin et al. 2002), and are now considered priority pollutants in the United States and Europe (US EPA 1999; European Union Commission 2001).

Di-esters are the most widely used class of plasticizer. This is often the phthalate di-ester, di-2-ethylhexyl phthalate (DEHP) (Cadogan et al. 1999; Wilson 1995; Ejlertsson et al. 1997),

but others such as di-2-ethylhexyl adipate (DEHA) are also commonly used (Tepper 1973; Startin et al. 1987; Nerin et al. 1992; Bohnert et al. 1999; Lang and Stanhope 2001). A study in Germany in 2002 found average DEHP levels of 2.27 μg/l in surface waters and 0.70 mg/kg in sediments (Fromme et al. 2002). Another report estimated median concentrations in rivers in the USA at 3 μg/l and 7 μg/l for DEHA and DEHP, respectively (Kobin et al. 2002).

Considerable work has been done in an attempt to assess the fate of these compounds in the environment as well as their ecological impacts (Cadogan et al. 1999; Cartwright et al. 2000; Fromme et al. 2002; Kolpin et al. 2002; Jonsson et al. 2003) and health risks (Cornu et al. 1992; Norback et al. 2000; Fukuwatari et al. 2002). Studies have shown that these compounds are degraded by certain microorganisms (Seager and Tucker 1973; Kurane et al. 1977; O'Grady et al. 1985; Cartwright et al. 2000; Jianlong et al. 2000). However, recent papers have shown that during the biodegradation of plasticizers by a variety of different microorganisms (Nalli et al. 2002; Gartshore et al. 2003), some of the intermediates can accumulate. These metabolites have also been observed in the environment (Jonsson et al. 2003; Horn et al. 2004). This is a problem because these metabolites were found to exhibit considerably higher acute toxicities than the original plasticizers (Nalli et al. 2002; Gartshore et al. 2003). Furthermore, some of these compounds have been linked to an increased frequency of asthma symptoms in hospitals (Norback et al. 2000), were identified as peroxisome proliferators in rats and mice (Cornu et al. 1992), and have been implicated in endocrine disruption (Fukuwatari et al. 2002).

The first step in the degradation pathway for DEHA (Fig. 1) was suspected to be the hydrolysis of one of the ester bonds, producing one molecule of 2-ethylhexanol and one of mono-2-ethylhexyl adipate, but this could only be inferred because the monoester was not observed (Nalli et al. 2002). Eventually, as observed in previous work (Nalli et al. 2002), both ester bonds in the monoester are hydrolyzed, leaving adipic acid and 2-ethylhexanol. The 2-ethylhexanol is further

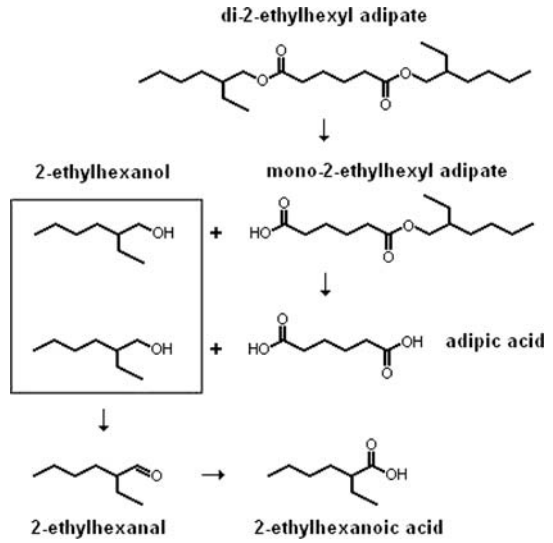


**Fig. 1** Diester plasticizer degradation pathway proposed by Nalli et al. (2002). Specific example shown is for DEHA

oxidized, but this often leads to an accumulation of the recalcitrant 2-ethylhexanoic acid.

Surfactants are also commonly present in the environment (Ding et al. 1999; Bodour et al. 2003; Zhu et al. 2003). They are released as industrial pollutants, through remediation strategies, and are produced indigenously by microorganisms. Their effects on the solubilization and biodegradation of hydrophobic compounds have been extensively studied (Prak and Pritchard 2002; Willumsen and Arvin 1999; Churchill et al. 1995; Zheng and Obbard 2001; Schippers et al. 2000; Ito and Inoue 1982; Oberbremer et al. 1990; Noordman et al. 2002; Deschenes et al. 1996; Guha and Jaffe 1996; Bramwell and Laha 2001), but these studies did not include plasticizers. The aim of the work presented here is to study the effect of some synthetic surfactants and biosurfactants on the microbial degradation of the plasticizers commonly found in the environment. The test microorganism, *Bacillus subtilis*, was selected because it can produce a very effective biosurfacant—surfactin. However, it is possible to obtain strains that do not produce this compound in order to allow experiments on the difference between degradation with and without the surfactant present.

## Materials and methods

### Chemicals

Di-2-ethylhexyl adipate (99% purity), 2-ethylhexanol (99.6% purity), 2-ethylhexanal (96% purity), 2-ethylhexanoic acid (99% purity) and *n*-pentadecane (99% purity) were all obtained from Sigma-Aldrich (Montreal, QC). The synthetic surfactants were Pluronic L122 (BASF Corp., Wyandotte, MI), Tween 80 (Atlas Chemical Industries, Brantford, ON), and Triton X-100 (Sigma-Aldrich, Montreal, QC). Glucose was obtained from American Chemicals Ltd. (Montreal, QC).

The biosurfactants were surfactin isolated from *B. subtilis* ATCC 21332 and sophorolipid from *C. bombicola* ATCC 22214. Surfactin was isolated and purified as described in Cooper et al. (1981). The lactone form of sophorolipid crystals was obtained as described in Cavalero and Cooper (2003). The biosurfactant crystals were stored at 4°C until needed. Prior to being added to microbial cultures, the crystals were re-suspended in Mineral salt medium (MSM) (Nalli et al. 2002) and filter-sterilized through 0.22 μm Millipore mixed-cellulose membrane filters (Fisher Scientific, Montreal, QC) using a vacuum filtration setup which was previously autoclaved for 1 h at 121°C and 220 kPa.

The critical micelle concentration (CMC) was obtained for each surfactant by suspending it in MSM and then performing serial dilutions with MSM over a wide range of surfactant concentrations. The surface tension measurements were made using the Wilhelmy plate method with a Krüss K12 processor-tensiometer (Krüss USA, Charlotte, NC).

### Cultures, growth conditions and fermentation

Strains of *B. subtilis* (ATCC 21332 & ATCC 6633) were obtained from American Type Culture Collections (ATCC) and were initially re-suspended as recommended by the supplier.

All shake flask cultures were grown in 500-ml Erlenmeyer flasks in a rotary shaker at 30°C at 150 rpm. The experimental media contained glucose at a concentration of 2.5 g/l in 100 ml MSM. Prior to inoculation, the MSM and concentrated glucose solution were autoclaved separately for 1 h at 120°C and 220 kPa. The glucose solution was then added to the flasks to obtain the required concentration. When used, plasticizer was added to cultures, prior to autoclaving, at a concentration of 2.5 g/l. Each surfactant was added at concentrations just above its critical micelle concentration. Synthetic surfactants were added before autoclaving, while biosurfactants were filter-sterilized then added with sterile syringes.

The above growth medium was used for cultures of *B. subtilis* ATCC 6633 in the 1-l sequencing batch cyclone reactor (Brown 2001). The reactor and the reservoir containing the medium were sterilized in an autoclave each for 3 h at 120°C and 220 kPa prior to inoculation. The contents of the reactor were continuously recirculated by a 1/55 hp centrifugal pump (1,750 rpm). The temperature of the broth was maintained at 30°C by means of a counter-current flow heat exchanger. A condenser at the top of the cyclone prevented volume loss due to evaporation. The cyclone was aerated through an in-line HEPA filter (Millipore Millex-FG50, 0.2 μl). Any liquid flow in or out of the reactor passed through isolators in order to prevent contamination. The separate components of the reactor were connected by latex tubing (VWR Scientific, West Chester, PA). The growth medium (2.5 g/l glucose in MSM) was supplied to the reactor by gravity from a reservoir. Cycling consisted of removing 90% of the reactor volume through the harvest port, then re-filling with fresh medium up to 1 l. Initial inoculation, plasticizer addition and surfactant solution addition (where used) were performed using sterile syringes through the septum of the injection port. A sample port equipped with an isolator was used to withdraw samples of 10 ml for analysis. Experiments were conducted to compare plasticizer degradation with and without surfactant addition. Surfactin is produced by various strains of *B. subtilis*, but other strains can be found that do not produce this biosurfactant. A strain (ATCC 6633) that could not produce surfactin was used in order to compare control experiments without the biosurfactant, to those in which surfactin, isolated from a producing strain (ATCC 21332) added.

## Analysis of biomass

A total of 100 μl were withdrawn from reactor samples or shake flasks for the assay. The quantification of microbial growth was performed using the BIORAD DC Protein Assay (BIO-RAD Laboratories, Hercules, CA) as described by BIO-RAD. Absorbance was measured at a wavelength of 750 nm using a Cary 50 Bio UV–visible spectrophotometer (Varian, Walnut Creek, CA). The results were compared to a standard curve prepared using bovine serum albumin and reported as g/l protein.

## Concentrations of plasticizers and their metabolites

Five milliliter of samples were withdrawn from the sample port. The pH was adjusted to below 2 with a drop of concentrated $H_2SO_4$ and an extraction was performed as above with 5 ml of the chloroform solution containing 1.5 g/l of n-pentane as an internal standard. The test tubes were vortexed and, after settling, samples of the organic phase were analyzed by GC.

The gas phase of the reactor was analyzed using solid phase micro-extraction (SPME). The 70 μm Carbowax/DVB StableFlex SPME fiber assembly (Supelco, Bellefonte, PA) with Manual Holder (Supelco, Bellefonte, PA) was inserted through a rubber septum in the head space of the reactor. The fiber was allowed to equilibrate for 30 min and was then inserted directly into the injector of the GC. A calibration curve was developed for 2-ethylhexanol in the gas phase by measuring its aqueous phase concentration during an abiotic reactor run ($r^2 = 0.98$, results not shown). The decrease in concentration due to evaporation over recorded time intervals was correlated to the peak size obtained from the SPME chromatograph.

Plasticizer and metabolite concentrations of organic extract samples were obtained using a Varian CP-3800 gas chromatograph (GC-FID) equipped with a CP-Sil five column (Varian, Walnut Creek, CA) with an internal diameter of 0.53 mm. The operating conditions were as follows: an injection temperature of 250°C; an initial column temperature of 60°C; a constant column flowrate of 10 ml/min; and a final column temperature of 300°C. The detector temperature was 300°C, while the temperature ramp rates were 10°C/min between 60°C and 150°C, followed by 20°C/min between 150°C and 300°C.

For the analysis of gas-phase 2-ethylhexanol samples obtained by SPME, the GC was loaded with a Varian fused silica CP SIL 8CB column with an internal diameter of 0.32 mm. The operating conditions were an injection temperature of 250°C; an initial column temperature of 60°C with a 1-min hold time; a ramp rate of 15°C/min; a final column temperature of 280°C with a 4-min hold time; a detector temperature of 300°C; and a column pressure of 8.3 kPa. The error in the data reported was ±0.02 g/l, as calculated from triplicate samples.

The peaks obtained by GC analysis were attributed to the various plasticizers and metabolites by comparison to standards. Where no commercial standard was available, identification was based on GC/MS analysis. The GC/MS (Thermo Quest model TRACE GC 2000/Finnigan POLARIS) was equipped with a Restek RTX-5 MS column (Fisher Scientific, Montreal, QC) with an internal diameter of 0.25 mm. The injection temperature was 250°C, the initial column temperature was 65°C, the ramp rate was 10°C/min, the final column temperature was 320°C, the final hold time was 2.5 min, the ramp hold time was 0.1 min, the MS start time was 2.2 min, the MS range was 50–600, the transfer line temperature was 275°C, and the ion source temperature was 200°C.

## Isolation and acute toxicity of mono-2-ethylhexyl adipate

The isolation of this monoester was performed after collecting the broth at the end of a fermentation to which surfactin was initially added. In order to ensure that any surfactin in the sample would remain in the aqueous phase, the monoester was extracted from the broth with chloroform at pH 7. The small amount of 2-ethylhexanol present was removed under reduced pressure along with the extraction solvent and this was verified by GC analysis. The end product was 95.6% pure as it contained a small amount of the original diester plasticizer.

The acute toxicity of mono-2-ethylhexyl adipate was measured using the Microtox toxicity assay, based on the toxic effect of the test compound on the respiration of the light-emitting marine organism *Vibrio fischeri* NRRL B-11177 (Bulich et al. 1981). An effective concentration that caused a 50% decrease in light output ($EC_{50}$) was obtained. The isolated mono-2-ethylhexyl adipate was re-suspended in MSM at 0.1 g/l and transferred into 0.5-ml cuvettes designed for the Microtox Model 500 (Microbics Corp., Carlsbaad, CA). The procedure then followed was according to the Basic Test setup in the Microtox software. The small amount of plasticizer contained in the sample would not interfere with the results because it causes minimal inhibition of the test organism (Nalli et al. 2002).

### Analysis of 2-ethylhexanol phase separation using microfiltration

Solutions of 1.0 mM 2-ethylhexanol and various concentrations of Pluronic L122 were prepared in MSM and the surface tension of these solutions was measured. A 5-ml sample of each solution was withdrawn and extracted with 5 ml of chloroform solution containing 1.5 g/l *n*-pentadecane as the internal standard. The extraction was performed inside a test tube, which was vortexed for 1 min. The initial 2-ethylhexanol concentration was determined by GC. Twenty milliliters of each solution was then vacuum filtered using 10-nm pore size nitro-cellulose membrane filters (Fisher Scientific, Montreal, Canada) for 20 min. The surface tension of the filtrate was measured and its 2-ethylhexanol concentration was obtained using the same method as for the initial solutions.

The membrane filters were also extracted to obtain an amount of 2-ethylhexanol retained on the filter. Following filtration, they were carefully placed in 40-ml capped glass vials and extracted with 10 ml of *n*-pentadecane in chloroform solution by manual agitation for 1 min. The concentration of the 2-ethylhexanol in the extract was then analyzed by GC as described above.

## Results

### Critical micelle concentrations

The critical micelle concentrations (CMC) were determined for the surfactants used in the growth studies. Table 1 summarizes these results and the concentrations that were added to the growth experiments.

### Effect of surfactin on plasticizer degradation by *B. subtilis* ATCC 6633

Fermentations were performed with *B. subtilis* ATCC 6633 in a 1-l reactor both with and without the addition of surfactin in order to observe its effects on DEHA degradation. As shown in Fig. 2, both the hydrolysis of DEHA and the growth of the organism occurred at approximately the same rate in the presence of surfactin as in the control experiment.

The profiles of metabolite concentrations versus time are shown in Fig. 3. 2-ethylhexanol reached dramatically higher concentrations when surfactin was present. Mono-2-ethylhexyl adipate reached a concentration in excess of 0.7 mM after surfactin was added, and remained in the medium

**Table 1** Critical micelle concentration (CMC) values of surfactants measured in the present study (and reported elsewhere) and concentrations added to fermentations

| Surfactant | CMC in MSM (g/l) | Reported CMC (g/l) | Concentration added (g/l) |
|---|---|---|---|
| Surfactin | 0.024 | 0.025[a] | 0.026 |
| Sophorolipid | 0.041 | 0.004[b]–0.082[c] | 0.045 |
| Triton X-100 | 0.070 | 0.043[d] | 0.077 |
| Tween 80 | 0.15 | 0.036[f] | 0.16 |
| Pluronic L122 | 0.15 | | 0.16 |

[a]Cooper et al. (1981); [b]Schippers et al. (2000); [c]Christofi and Ivshina (2002); [d]Guha and Jaffe (1996); [f]Zheng and Obbard (2001)

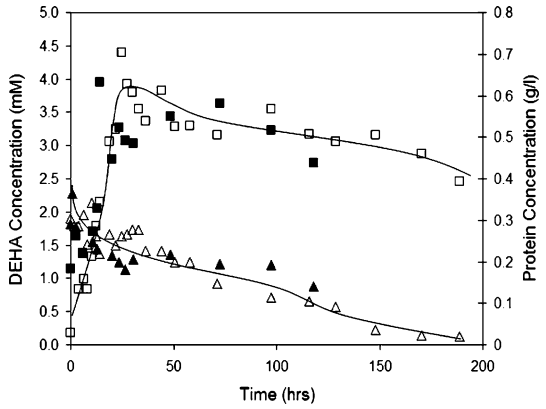


**Fig. 2** Growth of *B. subtilis* ATCC 6633 with (□) and without (■) surfactin present, and corresponding DEHA concentrations with (Δ) and without (▲) surfactin

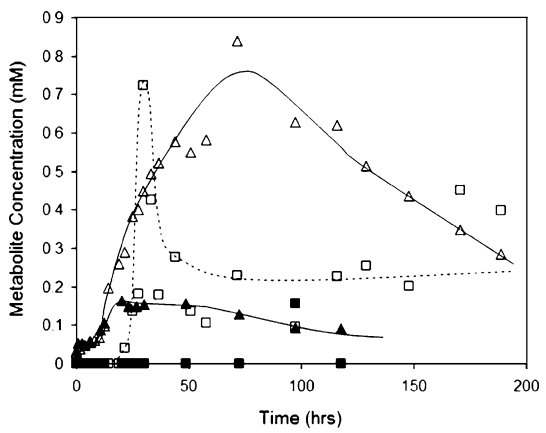


**Fig. 3** The concentrations of metabolites during the degradation of DEHA by *B. subtilis*. Hollow symbols represent the concentrations of 2-ethylhexanol (Δ) and mono-2-ethylhexyl adipate (□) in the presence of surfactin, while filled symbols represent the concentrations of 2-ethylhexanol (▲) and mono-2-ethylhexyl adipate (■) in a control experiment

at significant concentrations until the end of the fermentation. In the control fermentation, only one sample obtained late in the experiment contained a measurable amount of the monoester.

## Isolation and toxicity of mono-2-ethylhexyl adipate

Mono-2-ethylhexyl adipate was identified as a metabolite during the breakdown of DEHA by *B. subtilis* ATCC 6633 by GC/MS analysis, followed by an examination of the mass spectrum obtained for the unidentified peak. The identity and final purity of the isolated mono-2-ethylhexyl adipate was confirmed by GC/MS.

The toxicity of the isolated mono-2-ethylhexyl adipate was obtained using the Microtox acute toxicity assay. The result is tabulated in Table 2 along with the toxicities of the other DEHA metabolites obtained previously by Nalli et al. (2002) using the same method. Mono-2-ethylhexyl adipate was found to have a comparable toxicity to 2-ethylhexanol as expressed by an $EC_{50}$ on a molar basis.

## Effect of other surfactants on plasticizer degradation

The most dramatic effect of any of the other surfactants was observed for the addition of Pluronic L122. Its effect on metabolite concentrations in both aqueous and gas phases is shown in Fig. 4. However, no mono-2-ethylhexyl adipate was observed in these experiments. The addition of all of the other surfactants resulted in measurable amounts of the monoester that tended to accumulate in the medium. The maximum amounts observed were as follows: sophorolipid at 0.1 mM; Triton X-100 at 0.2 mM, and Tween 80 at 0.1 mM. In each of these experiments, 2-ethylhexanol also accumulated, but its concentration exceeded the control value only when sophorolipid was added (2.8 mM). None of the surfactants tested affected the growth of the organism.

## Interaction of 2-ethylhexanol with a surfactant

Figure 5 shows the increasing amount of 2-ethylhexanol that was extracted from membrane filters following the filtration of a 1 mM 2-ethylhexanol

**Table 2** The toxicities of DEHA metabolites as obtained with the Microtox assay

| Compound | $EC_{50}$ (mM) |
|---|---|
| 2-ethylhexanal | 0.703[a] |
| 2-ethylhexanol | 0.054[a] |
| 2-ethylhexanoic acid | 0.729[a] |
| Mono-2-ethylhexyl adipate | 0.079 |

[a]Nalli et al. (2002)

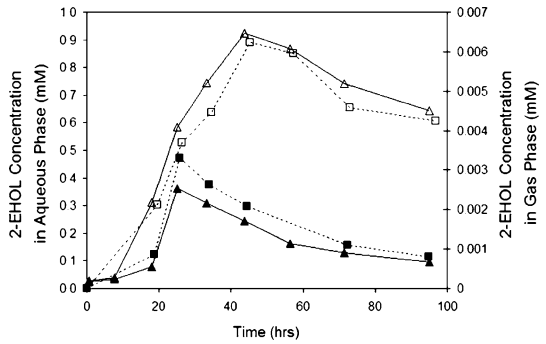


**Fig. 4** 2-ethylhexanol (2-EHOL) concentration during the growth of *B. subtilis* in the presence of DEHA in parallel reactors with and without Pluronic L122. Shown are the 2-ethylhexanol concentrations in the aqueous phase with (Δ) and without (▲) Pluronic L122 in the medium. The concentration in the gas phase is also shown with (□) and without (■) Pluronic L122 in the medium

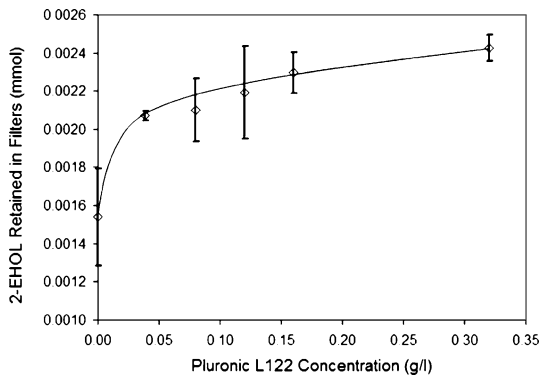


**Fig. 5** The amount of 2-ethylhexanol (2-EHOL) removed by micro-filtration from solutions of MSM containing different concentrations of Pluronic L122. Error bars indicate ±1 SD for triplicates

solution with increasing concentrations of Pluronic L122. It should be noted that some 2-ethylhexanol was always retained in the filter even without any surfactant added, but this amount increased significantly with surfactant present. The amount of the alcohol retained in the filters continued to increase gradually with more surfactant added, but the increase was less dramatic than the one observed for the first addition of the surfactant. Nevertheless, there was a significant difference between 0.16 g/l surfactant (i.e., the concentration added to cultures) and the lowest concentration tested at 0.04 g/l. The differences in the concentrations of 2-ethylhexanol between the initial solution and the filtrate followed the same trend as seen in Fig. 5 with increasing Pluronic L122 concentration (results not shown).

## Discussion

In earlier reports of the biodegradation of diester plasticizers, the monoester intermediates were inferred but not observed (Nalli et al. 2002). Thus it was not possible to determine their toxicities. In the present study, it was possible to obtain sufficiently large concentrations of one of these compounds, mono-2-ethylhexyl adipate, which allowed toxicity measurements to be made. With the exception of 2-ethylhexanol, which was of comparable toxicity, this compound was found to be an order of magnitude more toxic than any of the other metabolites tested (see Table 2). This result demonstrates the importance of any environmental situation that leads to significant amounts of the monoesters and 2-ethylhexanol being produced and accumulated.

Plasticizers are commonly found in the environment and significant concentrations of synthetic surfactants can also be present. The addition of surfactants to enhance the biodegradation of water insoluble compounds is a standard procedure (Churchill et al. 1995, Christofi and Ivshina 2002). Such an enhancement was expected to be observed during the first step of the biodegradation of the hydrophobic plasticizer, DEHA, upon surfactant addition. As well, many microorganisms commonly found in soil samples produce biosurfactants and these could also affect the metabolism of a sparingly soluble substrate such as DEHA. The addition of surfactin to cultures of *B. subtilis* did not, in fact, enhance the hydrolysis of the plasticizer, as was shown by an equal rate of plasticizer disappearance with and without the surfactant being present (see Fig. 2).

After the first hydrolysis step has occurred, the plasticizer fragments released (i.e., 2-ethylhexanol and mono-2-ethylhexyl adipate), as well as subsequent metabolites, are more water soluble than DEHA. It was therefore expected that the addition of a surfactant would not affect their biodegradation. However, the surfactin appeared to interfere with subsequent steps in

the biodegradation, resulting in significant early peaks in the concentrations of the intermediates 2-ethylhexanol and mono-2-ethylhexyl adipate (see Fig. 3). The concentrations of the alcohol and monoester remained elevated through to the end of the experiments, and no 2-ethylhexanoic acid was detected. This effect is particularly surprising for the monoester because this intermediate is usually not observed. For example, measurable amounts of mono-2-ethylhexyl adipate were not detected in the control experiment without the presence of surfactin.

Since biosurfactants are known to have a number of impacts on cells (Zhang and Miller 1994; Al-Tahhan et al. 2000; Ahimou et al. 2001; Kim et al. 2002), it is possible that the observed increase in metabolite accumulation was due to a specific interaction involving this lipopeptide biosurfactant within the enzymatic pathway responsible for plasticizer degradation. However, the same significant enhancement in the concentrations of the alcohol was observed with the synthetic surfactant, Pluronic L122. In these experiments, the monoester was not observed. However, experiments with the other surfactants (i.e., sophorolipid, Triton X-100 and Tween 80) did result in the accumulation of both the alcohol and the monoester. The structures of these three surfactants are all very different from each other and this suggests that a common enzymatic inhibition mechanism is unlikely.

Another possible cause of increased metabolite accumulation could have been an effect of the surfactant on the growth rate of the microorganism. However, none of the surfactants that caused increases in either 2-ethylhexanol or mono-2-ethylhexyl adipate concentrations caused any significant effect on the concentration of biomass. A possible effect by the surfactants on the partitioning of 2-ethylhexanol between the aqueous and gas phases was also important to consider. Figure 4 shows that the presence of surfactants did not affect alcohol volatilization, since the concentration of 2-ethylhexanol increased similarly in both phases when a surfactant was present.

While inhibition of the enzymes involved in the steps after the first hydrolysis step cannot be completely discounted, another possibility is that the observed phenomena are entirely due to a surfactant effect. This would be consistent with the fact that several different surface-active compounds were able to cause the large increase in the concentrations of the alcohol and monoester. Alcohols have been shown to interact closely with surfactants in an aqueous medium (Caragheorgheopol et al. 1997; Lin and Jiang 1997). In particular, they have been shown to form mixed micelles with surfactant molecules and subsequently change the surface chemistry of the mixture (Patist et al. 1998; Marangoni and Kwak 1991; Bockstahl and Duplatre 2001; Akhter and Al-Alawi 2000; Trotta et al. 1993; Su et al. 2003). For example, 2-ethylhexanol possesses antifoaming properties when added to some surfactant solutions (Jha et al. 1999). It has also been suggested that it interacts with bis-2-ethylhexyl sulfosuccinate micelles and affects the degree of micellar packing (Popovici and Chiriac 1994).

This leads to the hypothesis that the 2-ethylhexanol is being sequestered due to an interaction with the surfactants. This would reduce the effective concentration of this alcohol in the medium, which in turn would reduce the rate of enzymatic oxidation of 2-ethylhexanol to 2-ethylhexanoic acid. A similar sequestering phenomenon could be occurring with mono-2-ethylhexyl adipate. The structure of this monoester suggests that it could be surface active and thus it could also be incorporated into micelles or aggregates with the added surfactants.

In earlier work with Pluronic surfactants, filters with very fine pore sizes were used to retain surfactant aggregates (Lin et al. 2002; Schillen et al. 1999). This method was used to investigate the interaction of surfactants with the 2-ethylhexanol. These data have a large variation because the systems have several different phases and the potential for error is significant. However, the trend shown in Fig. 5 does clearly show that the interaction between the 2-ethylhexanol and the Pluronic L122 aggregates was strong enough to remove the alcohol from the solution.

A similar explanation was offered by Willumsen and Arvin (1999) for the increased accumulation of fluoranthene metabolites in media amended with Triton X-100 during the degradation of fluoranthene by *Sphingomonas paucimobilis*. It was shown that the addition of Triton X-100 to

the culture broth increased the rate of disappearance of fluoranthene, but decreased the overall extent of mineralization. The addition of the surfactant also caused an accumulation of an unidentified colored metabolite in the broth, which was not seen when surfactant was not added. It was suggested that although the presence of the surfactant improved the first step of fluoranthene degradation, it also created some form of inhibitory effect on the overall pathway that caused an intermediate product to accumulate. It was proposed that the metabolites released during the degradation of fluoranthene may have become associated with surfactant micelles due to the presence of hydroxy or carboxy groups, thereby causing them to become less bioavailable (Willumsen and Arvin 1999).

The present work has shown several important aspects of the first step in the degradation of DEHA. The kinetic studies performed in a well-mixed medium revealed that a suppression of plasticizer degradation subsequent to the first hydrolysis step can occur when a surfactant that sequesters one or more of the metabolites is added. Toxicity analyses showed that the metabolites produced by this first hydrolysis step, mono-2-ethylhexyl adipate and 2-ethylhexanol, are the most toxic intermediates in the plasticizer degradation pathway by an order of magnitude. Mono-2-ethylhexyl adipate was only shown to accumulate when a surfactant was present in the medium. Furthermore, while 2-ethylhexanol had been observed in previous studies (Nalli et al. 2002; Nalli et al. 2005), the presence of a surfactant significantly increased the concentration of this toxic metabolite in the medium. Surface active compounds are frequently found in the environment (Ding et al. 1999; Bodour et al. 2003; Zhu et al. 2003) and thus it seems reasonable to conclude that there will be situations in which significant amounts of mono-2-ethylhexyl adipate and 2-ethylhexanol could accumulate.

It is well established that plasticizers have become ubiquitous environmental contaminants. More recently, it has been shown that many microorganisms can partially degrade these compounds (Nalli et al. 2005), and the metabolites of partial plasticizer degradation (mono-2-ethylhexyl adipate, 2-ethylhexanol, 2-ethylhexanoic acid) were detected in several studies (Jonsson et al. 2003; Horn et al. 2004; Norback et al. 2000; Otake et al. 2001). Interestingly, the distribution of the metabolites varied dramatically between sampling sites. This work suggests that a possible factor affecting this distribution may be the presence of certain surface-active compounds. This is a particularly important consideration because the early metabolites exhibit much higher toxicities than the plasticizers and the metabolites further along the degradation pathway (Nalli et al. 2002; Gartshore et al. 2003).

**Acknowledgement** The financial support of the EJLB Foundation is gratefully acknowledged.

## References

Ahimou F, Jacques P, Deleu M (2001) Surfactin and iturin A effects on *Bacillus subtilis* surface hydrophobicity. Enzyme Microbial Technol 27(10):749–754

Akhter MS, Al-Alawi SM (2000) Influence of alcohols on the critical micelle concentration of non-aqueous micellar solutions. Colloids and surfaces, A. Physicochem Eng Aspects 164(2–3):247–255

Al-Tahhan RA, Sandrin TR, Bodour AA, Maier RM (2000) Rhamnolipid-induced removal of lipopolysaccharide from *Pseudomonas aeruginosa*: effect on cell surface properties and interaction with hydrophobic substrates. Appl Environ Microbiol 66(8):3262–3268

Bauer MJ, Herrmann R (1997) Estimation of the environmental contamination by phthalic acid esters leaching from household wastes. Sci Total Environ 208(1, 2):49–57

Bockstahl F, Duplatre G (2001) Effect of 1-pentanol on size and shape of sodium dodecyl sulfate micelles as studied by positron annihilation lifetime spectroscopy. J Phys Chem B 105(1):13–18

Bodour AA, Drees KP, Maier RM (2003) Distribution of biosurfactant-producing bacteria in undisturbed and contaminated arid southwestern soils. Appl Environ Microbiol 69(6):3280–3287

Bohnert T, Izadi R, Pitman S, Stanhope B (1999) Recent developments: benzoate esters in polyvinyl resilient flooring. J Vinyl Additive Technol 5(3):143–147

Bramwell D-AP, Laha S (2001) Effects of surfactant addition on the biomineralization and microbial toxicity of phenanthrene. Biodegradation 11(4):263–277

Brown WA (2001) The self-cycling fermentor – development, applications, and future opportunities. Recent Res Develop Biotechnol Bioeng 4:61–90

Bulich AA, Greene MW, Isenberg DL (1981) Reliability of the bacterial luminescence assay for determination of the toxicity of pure compounds and complex effluents. ASTM Special Tech Publ 737(Aquatic Toxicology and Hazard Assessment):338–347

Cadogan DF, Papez M, Poppe AC, Pugh DM, Scheubel J (1999) An assessment of the release, occurrence and possible effects of plasticizers in the environment. Prog Rubber Plastics Technol 10(1):1–19

Caragheorgheopol A, Caldararu H, Dragutan I, Joela H, Brown W (1997) Micellization and micellar structure of a poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) triblock copolymer in water solution, as studied by the spin probe technique. Langmuir 13(26):6912–6921

Cartwright CD, Thompson IP, Burns RG (2000) Degradation and impact of phthalate plasticizers on soil microbial communities. Environ Toxicol Chem 19(5):1253–1261

Cavalero DA, Cooper DG (2003) The effect of medium composition on the structure and physical state of sophorolipids produced by *Candida bombicola* ATCC 22214. J Biotechnol 103(1):31–41

Christofi N, Ivshina IB (2002) Microbial surfactants and their use in field studies of soil remediation. J Appl Microbiol 93(6):915–929

Churchill PF, Dudley RJ, Churchill SA (1995) Surfactant-enhanced bioremediation. Waste Manage (New York) 15(5/6):371–377

Cooper DG, Macdonald CR, Duff SJB, Kosaric N (1981) Enhanced production of surfactin from *Bacillus subtilis* by continuous product removal and metal cation additions. Appl Environ Microbiol 42(3):408–412

Cornu MC, Lhuguenot JC, Brady AM, Moore R, Elcombe CR (1992) Identification of the proximate peroxisome proliferator(s) derived from di-(2-ethylhexyl) adipate and species differences in response. Biochem Pharmacol 43(10):2129–2134

Deschenes L, Lafrance P, Villeneuve JP, Samson R (1996) Adding sodium dodecyl sulfate and *Pseudomonas aeruginosa* UG2 biosurfactants inhibits polycyclic aromatic hydrocarbon biodegradation in a weathered creosote-contaminated soil. Appl Microbiol Biotechnol 46(5–6):638–646

Ding W-H, Tzing S-H, Lo J-H (1999) Occurrence and concentrations of aromatic surfactants and their degradation products in river waters of Taiwan. Chemosphere 38(11):2597–2606

Ejlertsson J, Alnervik M, Jonsson S, Svensson BH (1997) Influence of water solubility, side-chain degradability, and side-chain structure on the degradation of phthalic acid esters under methanogenic conditions. Environ Sci Technol 31(10):2761–2764

European Union Commission (2001) Decision No. 2455/2001/EC of the European Parliament and of the Council of 20 November 2001 establishing the list of priority substances in the field of water policy and amending Directive 2000/60/EC. Official Journal of European Communities L 331 1

Fromme H, Kuchler T, Otto T, Pilz K, Muller J, Wenzel A (2002) Occurrence of phthalates and bisphenol A and F in the environment. Water Res 36(6):1429–1438

Fujii M, Shinohara N, Lim A, Otake T, Kumagai K, Yanagisawa Y (2003) A study on emission of phthalate esters from plastic materials using a passive flux sampler. Atmos Environ 37(39–40):5495–5504

Fukuwatari T, Suzuki Y, Sugimoto E, Shibata K (2002) Identification of a toxic mechanism of the plasticizers, phthalic acid esters, which are putative endocrine disrupters: time-dependent increase in quinolinic acid and its metabolites in rats fed di(2-ethylhexyl)phthalate. Biosci Biotechnol Biochem 66(12):2687–2691

Gartshore J, Cooper DG, Nicell JA (2003) Biodegradation of plasticizers by *Rhodotorula rubra*. Environ Toxicol Chem 22(6):1244–1251

Graham PR (1973) Phthalate ester plasticizers. Why and how they are used. Environ Health Perspect 3:3–12

Guha S, Jaffe PR (1996) Biodegradation kinetics of phenanthrene partitioned into the micellar phase of nonionic surfactants. Environ Sci Technol 30(2):605–611

Hill SS, Shaw BR, Wu AHB (2003) Plasticizers, antioxidants, and other contaminants found in air delivered by PVC tubing used in respiratory therapy. Biomed Chromatogr 17(4):250–262

Horn OJ, Nalli S, Cooper DG, Nicell JA (2004) Plasticizer metabolites in the environment. Water Res 38(17):3693–3698

Ito S, Inoue S (1982) Sophorolipids from *Torulopsis bombicola*: possible relation to alkane uptake. Appl Environ Microbiol 43(6):1278–1283

Jha BK, Patist A, Shah DO (1999) Effect of antifoaming agents on the micellar stability and foamability of sodium dodecyl sulfate solutions. Langmuir 15(9):3042–3044

Jianlong W, Lujun C, Hanchang S, Yi Q (2000) Microbial degradation of phthalic acid esters under anaerobic digestion of sludge. Chemosphere 41(8):1245–1248

Jonsson S, Ejlertsson J, Ledin A, Mersiowsky I, Svensson BH (2003) Mono- and diesters from *o*-phthalic acid in leachates from different European landfills. Water Res 37(3):609–617

Kim K, Yoo D, Kim Y, Lee B, Shin D, Kim EK (2002) Characteristics of sophorolipid as an antimicrobial agent. J Microbiol Biotechnol 12(2):235–241

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. Environ Sci Technol 36(6):1202–1211

Kurane R, Suzuki T, Takahara Y (1977) Microbial degradation of phthalate esters. Part I. Isolation of microorganisms growing on phthalate esters and degradation of phthalate esters by *Pseudomonas acidovorans* 256–1. Agric Biol Chem 41(11):2119–2123

Lang J, Stanhope BE (2001) Benzoate plasticizer for flexible PVC injection molded toy applications. Plastics Additives Compounding 3(6):30–33

Lin S-C, Jiang H-J (1997) Recovery and purification of the lipopeptide biosurfactant of *Bacillus subtilis* by ultrafiltration. Biotechnol Techniq 11(6):413–416

Lin Y, Smith TW, Paschalis A (2002) Adsorption of amphiphilic copolymers on hydrophobic particles in aqueous media. J Dispersion Sci Technol 23(4):539–553

Marangoni DG, Kwak JCT (1991) Solubilization of alcohols and ethoxylated alcohols in anionic and cationic micelles. Langmuir 7(10):2083–2088

Nalli S, Cooper DG, Nicell JA (2002) Biodegradation of plasticizers by *Rhodococcus rhodochrous*. Biodegradation 13(5):343–352

Nalli S, Horn OJ, Grochowalski AR, Cooper DG, Nicell JA (2005) Origin of 2-ethylhexanol as a VOC. Environ Pollut 140:181–185

Nerin C, Gancedo P, Cacho J (1992) Determination of bis(2-ethylhexyl) adipate in food products. J Agric Food Chem 40(10):1833–1835

Norback D, Wieslander G, Nordstrom K, Walinder R (2000) Asthma symptoms in relation to measured building dampness in upper concrete floor construction, and 2-ethyl-1-hexanol in indoor air. Int J Tuberculosis Lung Dis 4(11):1016–1025

Noordman WH, Wachter JHJ, de Boer GJ, Janssen DB (2002) The enhancement by surfactants of hexadecane degradation by *Pseudomonas aeruginosa* varies with substrate availability. J Biotechnol 94(2):195–212

Oberbremer A, Mueller-Hurtig R, Wagner F (1990) Effect of the addition of microbial surfactants on hydrocarbon degradation in a soil population in a stirred reactor. Appl Microbiol Biotechnol 32(4):485–489

O'Grady DP, Howard PH, Werner AF (1985) Activated sludge biodegradation of 12 commercial phthalate esters. Appl Environ Microbiol 49(2):443–445

Otake T, Yoshinaga J, Yanagisawa Y (2001) Analysis of organic esters of plasticizer in indoor air by GC-MS and GC-FPD. Environ Sci Technol 35(15):3099–3102

Patist A, Axelberd T, Shah DO (1998) Effect of long chain alcohols on micellar relaxation time and foaming properties of sodium dodecyl sulfate solutions. J Colloid Interface Sci 208(1):259–265

Peakall DB (1975) Phthalate esters: occurrence and biological effects. Residue Rev 54:1–41

Popovici H, Chiriac A (1994) Co-surfactant effect of 2-ethylhexanol resulting from (bis 2-ethyl) sulfosuccinate sodium salt alkaline hydrolysis on surfactant micellar structure. Analele Universitatii de Vest din Timisoara. Seria Chimie 3:89–98

Prak DJL, Pritchard PH (2002) Degradation of polycyclic aromatic hydrocarbons dissolved in Tween 80 surfactant solutions by *Sphingomonas paucimobilis* EPA 505. Can J Microbiol 48(2):151–158

Saeger VW, Tucker III ES (1973) Phthalate esters undergo ready biodegradation. Plastics Eng 29(8):46–49

Schillen K, Bryskhe K, Mel'nikova YS (1999) Vesicles formed from a poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) triblock copolymer in dilute aqueous solution. Macromolecules 32(20): 6885–6888

Schippers C, Gessner K, Muller T, Scheper T (2000) Microbial degradation of phenanthrene by addition of a sophorolipid mixture. J Biotechnol 83(3):189–198

Staples CA, Peterson DR, Parkerton TF, Adams WJ (1997) The environmental fate of phthalate esters: a literature review. Chemosphere 35(4):667–749

Startin JR, Sharman M, Rose MD, Parker I, Mercer AJ, Castle L, Gilbert J (1987) Migration from plasticized films into foods. 1. Migration of di-(2-ethylhexyl)adipate from PVC films during home-use and microwave cooking. Food Additives Contaminants 4(4):385–398

Su Y-L, Wei X-F, Liu H-Z (2003) Influence of 1-pentanol on the micellization of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) block copolymers in aqueous solutions. Langmuir 19(7):2995–3000

Tepper LB (1973) Phthalic acid esters – an overview. Environ Health Perspect 3:179–182

Trotta M, Gasco MR, Pattarino F (1993) Effect of a series of alcohols on critical micellar concentration of hexyl phosphate: a quantitative structure-CMC relationship study. J Colloid Interface Sci 158(1):133–135

US Environmental Protection Agency (1999) Introduction to water policy standards. Office of Water, Washington, DC

Willumsen PA, Arvin E (1999) Kinetics of degradation of surfactant-solubilized fluoranthene by a *Sphingomonas paucimobilis*. Environ Sci Technol 33(15):2571–2578

Wilson AS (1995) Plasticizers: principles and practice. Institute of Materials, London

Zhang Y, Miller RM (1994) Effect of a *Pseudomonas* rhamnolipid biosurfactant on cell hydrophobicity and biodegradation of octadecane. Appl Environ Microbiol 60(6):2101–2106

Zheng Z, Obbard JP (2001) Effect of non-ionic surfactants on elimination of polycyclic aromatic hydrocarbons (PAHs) in soil-slurry by *Phanerochaete chrysosporium*. J Chem Technol Biotechnol 76(4):423–429

Zhu L, Yang K, Lou B, Yuan B (2003) A multi-component statistic analysis for the influence of sediment/soil composition on the sorption of a nonionic surfactant (Triton X-100) onto natural sediments/soils. Water Res 37(19):4792–4800