

MICROBIAL DEGRADATION OF LINEAR ALKYLBENZENESULFONATES IN SEWAGE CONTAMINATED GROUNDWATER

BRR406

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INTRODUCTION

Surfactants, or surface active agents, are the active ingredients in detergents used for domestic cleaning applications, such as dish washing and laundering. They are effective cleaning agents by concentrating at interfacial regions, lowering the surface tension of water, and forming micelles that solubilize oils (West, 1992). Surfactants are amphiphilic, because they are composed of both hydrophilic and hydrophobic moieties. The hydrophobic moiety interacts with non-polar phases, such as oils, while the hydrophilic moiety interacts with polar phases, such as water. Oils are removed from surfaces by partitioning into the hydrophobic interior of the surfactant micelle. Upon rinsing with water, the micelles carrying the oils are washed away to waste water treatment facilities, septic tanks, or drain fields.

Linear alkybenzenesulfonates (LAS) are the main anionic surfactants used in detergent formulations (Figure 1). LAS is a mixture of homologues that range between 10 and 14 carbon atoms on the aliphatic chain (Swisher, 1987). Each LAS homologue consists of different isomers, defined by the position of the phenyl ring on the aliphatic chain. The homologues and isomers have different sorption and biodegradation characteristics. For example, Schöberl (1989) found that longer-chain LAS homologues and isomers biodegrade faster than shorter-chain LAS homologues and isomers. Hand and Williams (1987) found that longer LAS alkyl-chain homologues and isomers have higher sediment partition coefficients than their shorter chain

counterparts. LAS also is generally considered to be biodegradable in aerobic environments and persistent in anaerobic environments (Leisinger et al., 1981). The reason LAS biodegradation is limited to aerobic conditions is because molecular oxygen is required for the first step in its biodegradation pathway.

As LAS is a constituent of detergents used in everyday cleaning, it is found in most municipal sewage effluents. Because LAS is an anthropogenic chemical and does not occur naturally, it is a good indicator of groundwater contamination (LeBlanc, 1984). An excellent site for the study of LAS behavior in groundwater is the U.S. Geological Survey's (USGS) Cape Cod Toxic Water Research Site, located on Cape Cod, Massachusetts, where sewage effluent from a waste water treatment facility is discharged onto a shallow groundwater aquifer (LeBlanc, 1984) (Figure 2). The groundwater moves at a rate of 0.2 to 0.7 m/day and has a temperature range of 9.5 to 14°C (Harvey et al., 1994). The waste water treatment facility, located on the Otis Air National Guard Base, has discharged secondary sewage effluent onto 4.86 hectares of rectangular sand beds since 1936 (LeBlanc, 1984). The result of this long-term sewage discharge is a large cigar-shaped that is currently 4000 m long, 762 to 914 m wide and 23 m thick. The plume contains nitrate, ammonia, LAS, chloride, boron, sulfate, and phosphate (LeBlanc, 1984). In addition, alkylbenzenesulfonate (ABS), a branched-chain non-biodegradable analog of LAS, was discharged in the sewage effluent until 1964 and is found in the farthest down-gradient (2-3 km) portion of the plume (LeBlanc, 1984). After 1964, LAS replaced ABS because LAS is more readily biodegradable.

Although metabolites of LAS have been detected in the plume, Field et al, (1992) estimated the rate at which LAS is biodegraded to be slow as LAS was detected after a 2.7 to 4.6

year residence time in the sewage contaminated groundwater. Low temperature (9.5 to 14°C) and oxygen conditions (<1 mg/L) in the groundwater are thought to hinder the biodegradation of LAS (Barber et al., 1995). Because LAS biodegradation has not been fully characterized in the Cape Cod site, research was aimed at characterizing LAS biodegradation at 25°C and 10°C in lab incubations with groundwater collected from the Cape Cod site.

MATERIALS and METHODS

Chemicals

A commercial mixture of LAS was obtained from Vista Chemical Company. The mixture was 50.05% active and was composed of 21.8% C₁₀-LAS, 39.1% C₁₁-LAS, 28.4% C₁₂-LAS, and 8.5% C₁₃-LAS homologues. A 99% pure C₈-LAS (4-octylbenzenesulfonic acid), received from Lancaster Synthesis Ltd., (Lancaster, England) was used as a surrogate standard. A 99% pure C₁₄-SAS (*n*-tetradecanesulfonic acid), obtained from the Aldrich Chemical Co., (Milwaukee, WI) was used as an instrumental standard. Both the surrogate and instrumental standards were chosen because they do not occur in commercial LAS mixtures. A four carbon-alkyl chain sulfophenyl carboxylated metabolite (SPC-4) of LAS was used as a surrogate standard for LAS metabolite quantification because it does not occur as a natural metabolite of LAS biodegradation. The mineral salts solution used in the turbidity experiments was made from 1 L deionized H₂O, 0.5 g NH₄NO₃, 1.3 g K₂HPO₄, 0.05 g CaCl₂, 0.2 g MgSO₄, 3.3 mL ethylenediaminetetraacetic acid (EDTA), 1 mL FeCl₃, with no trace elements or vitamins added.

Sterilization

All equipment used in the turbidity and incubation experiments was sterilized by autoclaving at 120°C and 15 psi for 30 minutes. For sterile controls, sediment core samples and groundwater samples also were autoclaved at the above settings. Solutions of LAS were filtered through a 0.45 µm filter and stored at 4°C until used. All sampling procedures were performed in a positive air flow hood with gloves worn to control for potential contamination. The turbidity flasks and the incubation tubes were all plugged with cotton wrapped in cheese cloth and covered with aluminum foil to insure that the experiments were aerobic, while keeping contaminants out.

Sediment Core Samples

The sediment core samples used in the turbidity experiment were taken from the USGS Cape Cod site near the area where previous tracer tests were performed in 1993, 1994 and 1995 (Barber et al., 1995). The sediment cores were taken from an interval between 7.7 to 8.0 m below land surface. The cores were packed in ice and sent to Oregon State University, where they were stored at 4°C.

Groundwater Samples

Groundwater samples used in the turbidity and incubation experiments were obtained from the Cape Cod site from 7.9 m below land surface. The groundwater samples were collected with a Geotech peristaltic pump fitted with Norprene tubing at a pumping rate of about 500 mL/min. The lines were purged with groundwater prior to sample collection. Prior to filling, glass collection bottles also were rinsed with groundwater. The bottles were then filled, capped and shipped on ice to OSU where they were stored at 4°C.

Turbidity Experiments

Turbidity experiments were performed on the sediment core and groundwater samples from the Cape Cod site as a crude method of estimating the lag time prior to LAS biodegradation at room temperature. Two different turbidity experiments were run. The first turbidity experiment was performed on the sediment core samples with mineral salts solution to investigate the potential for sediment-bound microorganisms to biodegrade LAS. First, approximately 3.5 g of sediment core was placed in four 250 mL Erlenmeyer flasks containing 20 mL of the mineral salts solution. Volumes of LAS solution were spiked into the four flasks to give final concentrations of 13, 65, 131 and 261 mg/L LAS. This procedure was done in triplicate to yield a total of twelve flasks with two replicate runs (A and B) with four flasks each, and a sterile control consisting of four flasks. The flasks were then capped, as described in the sterilization section, and set on a desktop to incubate at 25°C. The flasks were swirled daily to promote uniform LAS distribution. Daily observations for turbidity were made by holding the flasks up to light.

The second turbidity experiment was performed with groundwater from the Cape Cod site to investigate the potential for free living microorganisms to biodegrade LAS. For this experiment, 20 mL of groundwater were added to four 250 mL Erlenmeyer flasks. To two of the flasks, approximately 3.5 g of sediment core was added, while the remaining two had no sediment. Next, volumes of LAS solution were spiked into the groundwater and the groundwater plus sediment core flasks to give final concentrations of 13 and 65 mg/L LAS. This procedure was done in duplicate to yield eight flasks, one run with four flasks consisting of both the groundwater and the groundwater plus sediment core flasks and a sterile control consisting of

four flasks. The flasks were treated thereafter as described in the first turbidity experiment.

Groundwater Incubations

Ground water incubations with LAS were performed aerobically at both 25°C and 10°C to test the effect that temperature has on LAS biodegradation. The 25°C incubations were set up in nine 80 mL glass tubes. The tubes were organized into two replicate runs of three tubes each and a sterile control with three tubes. First, 60 mL of groundwater was added to all nine tubes. Volumes of LAS solution were spiked into the tubes to give final concentrations of about 15 mg/L. The tubes were capped, as described in the sterilization section, and set on a desktop to incubate at 25°C. Samples for LAS and LAS metabolites were taken daily until day 26. Thereafter, samples were taken every other day to conserve the remaining solution.

The 10°C incubations were set up in a manner similar to that for the 25°C incubations, except that eight tubes were placed in a cold water bath at 10°C to incubate. The incubations were organized with a sample run with four tubes, and a sterile control with four tubes. Samples were taken every other day until day twelve. After day twelve, samples were taken every fifth day because the loss of LAS was taking longer than expected. After 46 days, samples were again taken every other day because the loss of LAS was nearly complete. At day 69, samples were taken every four days to make the experiment last as long as possible with the amount of sample remaining.

Sample Extraction

Sample extraction for the 25°C groundwater incubation experiment consisted of taking 3 mL aliquots from each tube in each run. This yielded a composite sample of 9 mL for each run. The extracted samples were preserved immediately with 3% formalin (v/v) and stored at 4°C until

analyzed. Sample extraction for the 10°C groundwater incubation experiment was performed in a manner similar to the extraction procedure for the 25°C, except that 2 mL aliquots were taken from each tube in each run, yielding a composite sample of 8 mL for each run.

Instrumentation

A gas chromatograph with a flame ionization detector (GC-FID) was used for the specific detection, identification and quantitation of LAS and its homologues (Krueger and Field, 1995). LAS was quantified by summing the areas under the peaks between 15.5 and 18.6 minutes (Figure 3). The total area was then divided by the peak area corresponding to C₈-LAS. The concentration of LAS was then calculated from a linear calibration curve. LAS homologues were quantified by choosing one isomer to represent each homologue (Figure 3). The isomers picked to represent the homologues were chosen because they did not co-elute with other isomers. Quantitation was done in the same manner as that for total LAS. LAS metabolites were derivatized to their trifluoroethyl derivatives and analyzed using GC coupled with a negative chemical ionization mass spectrometer (GC-NCI/MS) (Field et al., 1992).

RESULTS

Turbidity

In the first turbidity experiment, where only sediment core and mineral salts solution was used, no turbidity was observed in the first 21 days. Because 21 days was longer than was expected for LAS loss to be seen, 2 mL aliquots of the 13 mg/L turbidity flasks and 1 mL aliquots of the 65 mg/L turbidity flasks were taken out and tested by GC-FID to see if there was LAS loss. The 13 mg/L turbidity flasks showed almost complete LAS loss. However, the 65 mg/L turbidity

flasks and the 13 and 65 mg/L sterile control flasks showed no LAS loss. The same analytical procedure was performed again on the 26th day of the experiment to check the status LAS loss in the 13 and 65 mg/L turbidity flasks. The 13 mg/L turbidity flasks showed no LAS remaining in solution, whereas, the 65 mg/L turbidity flasks and both the 13 and 65 mg/L sterile control flasks again showed no LAS loss. The 65 mg/L turbidity flasks were checked again on day 55 of the experiment, but still no LAS loss was observed.

In the second turbidity experiment, where groundwater only and groundwater plus sediment core was used, a lack of surfactancy (foaming) was noticed upon swirling in the 13 mg/L turbidity flasks. As a lack of surfactancy can be an indication of LAS biodegradation, 3 mL aliquots were taken from each of the 13 mg/L turbidity flasks and tested qualitatively by GC-FID for LAS loss. The 13 mg/L turbidity flasks showed almost complete loss of LAS, but the sterile controls remained stable. There was no difference in LAS loss between the groundwater only and the groundwater plus sediment core turbidity flasks. The 65 mg/L turbidity flasks were analyzed qualitatively on days 35 and 55 of the experiment, however no LAS loss was observed.

Turbidity was never observed in any of the turbidity flasks with or without the sediment core. The lack of turbidity is most likely due to small populations of microorganisms in both the sediment core and the groundwater samples. This hypothesis is plausible given that the samples came from a sandy, dark, cold (9.5 to 14°C) groundwater system with low (<1 mg/L) dissolved oxygen conditions. For turbidity to be an effective gauge of microbial growth, large populations of microbes are needed. The data gathered from the turbidity experiments did, however, give an estimation for the lag time for LAS biodegradation. The lag time estimated from the turbidity experiments at 25°C was between 13 and 21 days for concentrations of 13 mg/L LAS. There was

a lag time difference between the sediment core plus mineral salts solution and the groundwater turbidity experiments. This difference could be due to lower active microbial populations in the sediment core plus mineral salts solution than in the groundwater turbidity flasks. This is explained by remembering that the first turbidity experiment contained sediment core and mineral salts solution only, whereas the second experiment contained groundwater only, and groundwater plus sediment core. Since there was no difference between the groundwater only and the groundwater plus sediment core, but there was a difference between sediment core and mineral salts and groundwater, the groundwater must contain the more active microbial population.

25°C Groundwater Incubations

LAS biodegradation began after a ten day lag period in the 25°C groundwater incubations (Figure 4). By the last day of the incubations (day 37), 98.9% of the LAS in solution had biodegraded (Figure 5). Biodegradation affected the longest LAS alkyl-chain homologues and isomers first followed by the shorter LAS alkyl-chain homologues and isomers, which is in agreement with that reported by Schöberl (1989). Disappearance curves for LAS homologues are shown in Figure 6. The C₁₃ homologue disappeared on day 18 of the incubations. The C₁₂ and C₁₁ homologues both disappeared on day 21 followed by the C₁₀ homologue on day 22. The isomers chosen to represent the homologues gave a good approximation of the order of biodegradation. Note that not all isomers of each homologue disappeared on the days indicated, only the selected isomers, as shown in Figure 5. At the end of the experiment there was 0.2 mg/L LAS remaining in solution. The remaining LAS was composed primarily of the 5-phenyl-C₁₀ isomer, where the phenyl ring is attached to the fifth carbon atom of the C₁₀ alkyl-chain homologue, indicating that the 5-phenyl-C₁₀ LAS homologue is the most persistent.

Even though LAS metabolites were detected in the incubations, mass balance was not achieved (Figure 7). By the end of the incubations, almost all of the LAS had biodegraded (98.9%), but metabolites accounted for only 20.6% of the missing LAS mass. This lack of mass balance indicates that LAS may be completely mineralized to CO₂ under the conditions of this experiment. Throughout the duration of the experiment, none of the sterile controls showed any evidence of LAS biodegradation or metabolite formation.

10°C Groundwater Incubations

LAS biodegradation began after about a 32 day lag period in the 10°C incubations (Figure 8). After a total of 84 days, 89% of the LAS in solution had biodegraded (Figure 9). LAS homologues disappeared just as in the 25°C incubations with the longer alkyl-chain LAS homologues and isomers disappearing before the shorter alkyl-chain LAS homologues and isomers (Figure 10). The C₁₃ homologue disappeared by day 37 of the incubations. The C₁₂ and C₁₁ LAS homologues disappeared by day 42, followed by the C₁₀ LAS homologue on day 46. Again, not all isomers of the LAS homologues disappeared on the days indicated, just the isomer that was chosen to represent each homologue, as shown in Figure 9. At the end of the experiment there was 1.7 mg/L LAS remaining in solution. The remaining LAS was composed of 4 and 5-phenyl C₁₀ isomers, as well as the 5-phenyl C₁₁ isomer. The remaining isomers are the two shortest isomers of the C₁₀ homologue and the shortest isomer of the C₁₁ homologue. This data is in agreement with the 25°C incubations and the data reported by Schöberl (1989).

LAS metabolite formation was observed during the experiment, and again, mass balance was not achieved (Figure 11). During the experiment, 89% of the LAS in solution was biodegraded, however only 7.8% was recovered in the form of LAS metabolites. This lack of

mass balance is again evidence that LAS may be almost completely mineralized to CO₂ under the conditions of this experiment. The sterile controls for this experiment also remained stable, with neither LAS loss nor metabolite formation.

CONCLUSIONS

The research in this paper confirms that LAS is biodegradable under aerobic conditions. The lag time for LAS biodegradation was between 13 and 21 days in the turbidity experiments at 25°C with 13 mg/L LAS. At 25°C, groundwater incubations gave a lag time of 10 days, while the 10°C groundwater incubations gave a lag time of 32 days, with 15 mg/L LAS. The two and a half times difference in lag times between the 10°C and the 25°C experiments is in accordance with the Q-10 effect, which predicts that the rate of biodegradation should double for every 10°C difference in temperature. The microorganisms present in the groundwater preferentially biodegraded the longer LAS alkyl-chain homologues and isomers before the shorter LAS alkyl-chain homologues and isomers. The reason that the shorter LAS alkyl-chain homologues and isomers are more persistent than the longer LAS alkyl-chain homologues and isomers is most likely due to the close proximity of the aromatic moiety to the terminal carbon of the shorter alkyl-chains which limits the rate of enzymatic biodegradation of the shorter LAS alkyl-chain homologues and isomers making them more persistent (Swisher, 1978).

LAS metabolites were formed during the biodegradation of LAS but mass balance was not achieved. The lack of mass balance is evidence that LAS may be mineralized to CO₂ during biodegradation. To verify that mineralization is indeed occurring, CO₂ formation could be measured. Finally, the absence of LAS biodegradation in the turbidity experiments in concentrations higher than 13 mg/L shows that high concentrations of LAS (> 13 mg/L) may be

either inhibitory or toxic to the groundwater microorganisms.

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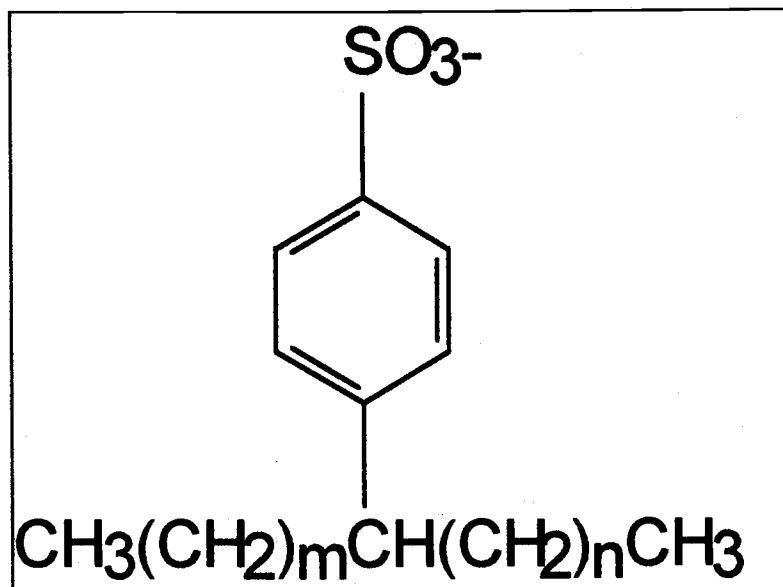


Figure 1: General structure for LAS. $m + n = 7-11$

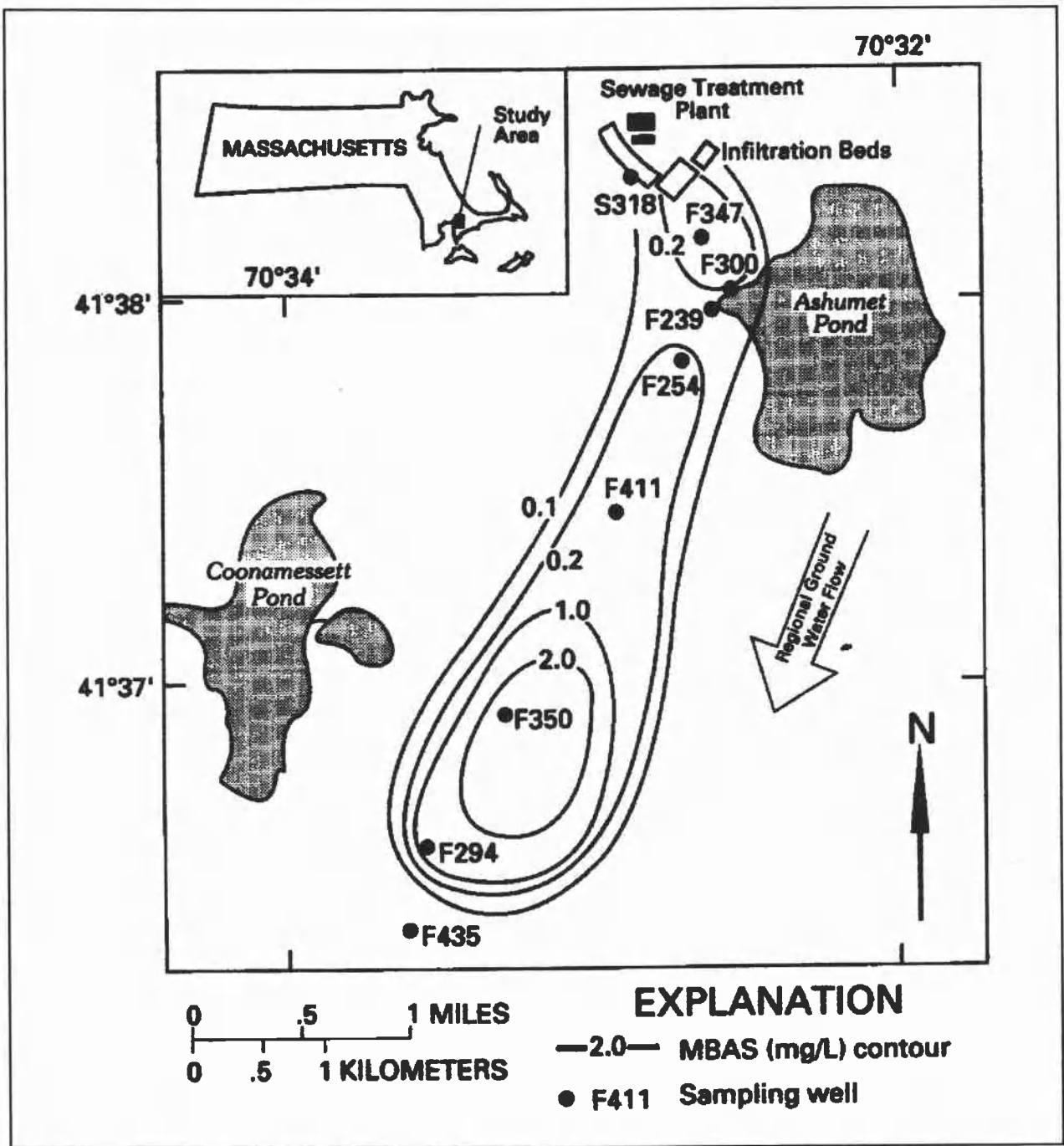


Figure 2: Cape Cod sewage plume.

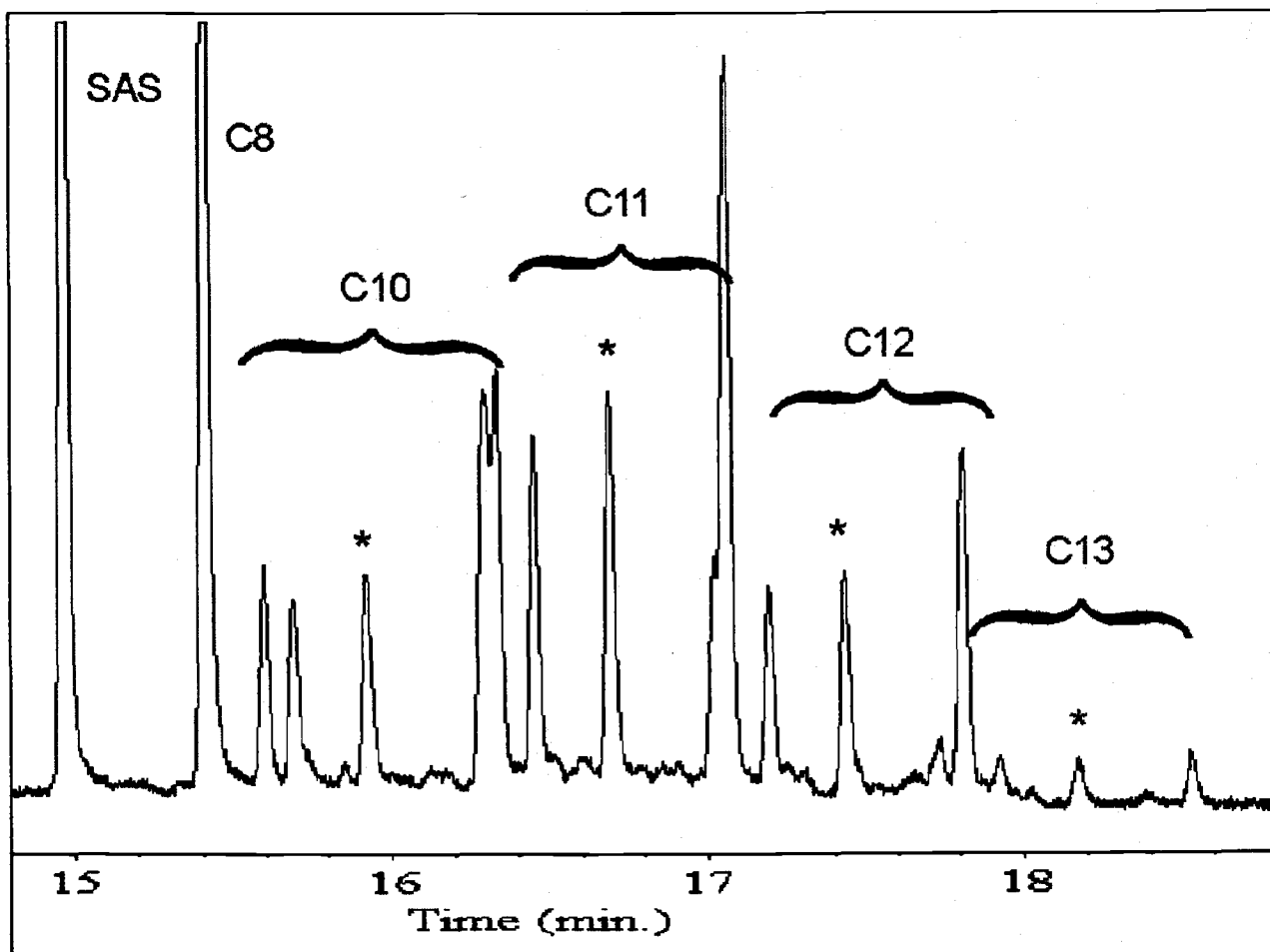


Figure 3: Standard chromatogram of LAS indicating the various homologues and the isomers chosen (*) to represent each homologue. SAS is the instrumental standard, and C8 is the surrogate standard.

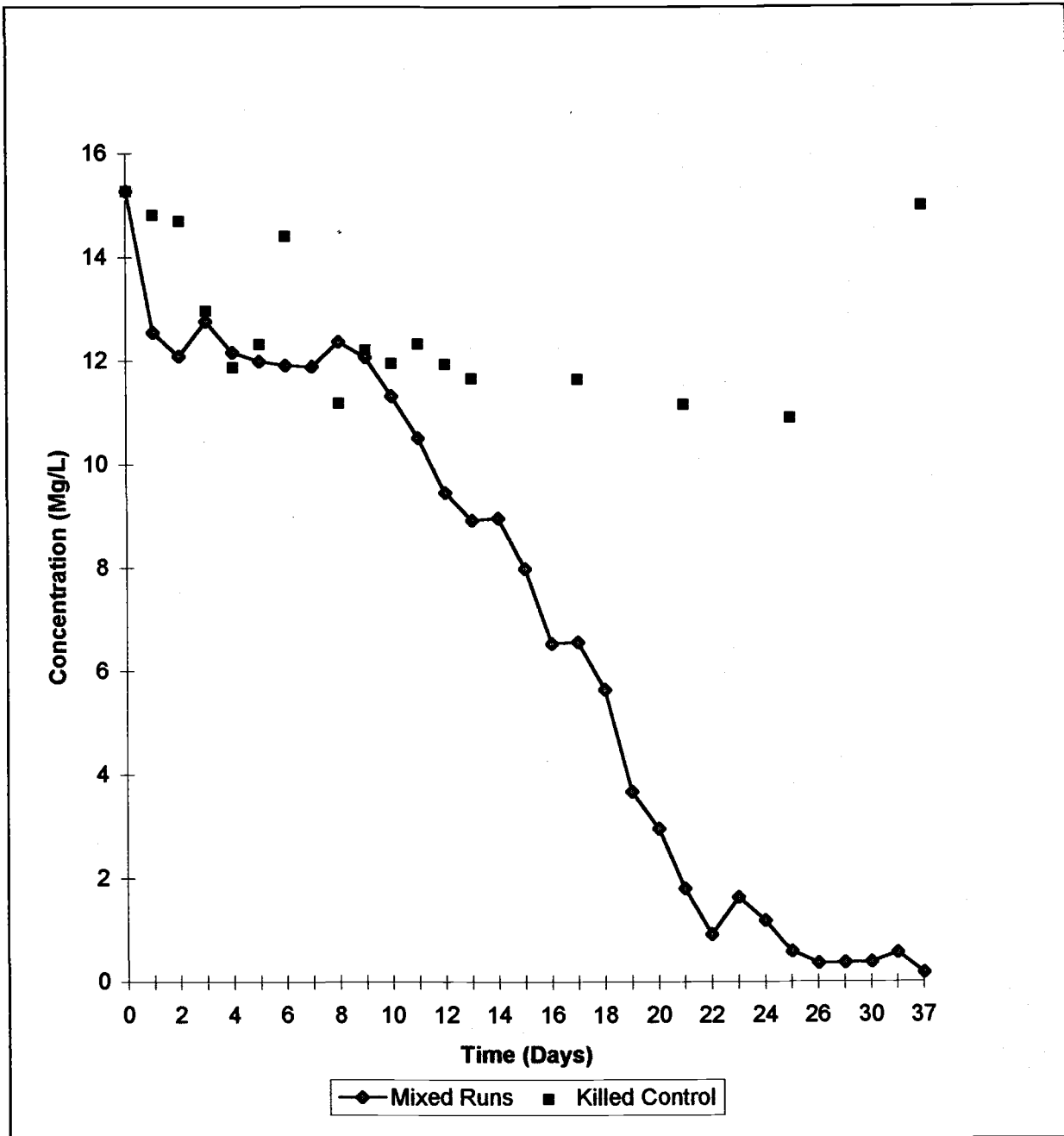


Figure 4: LAS loss at 25°C.

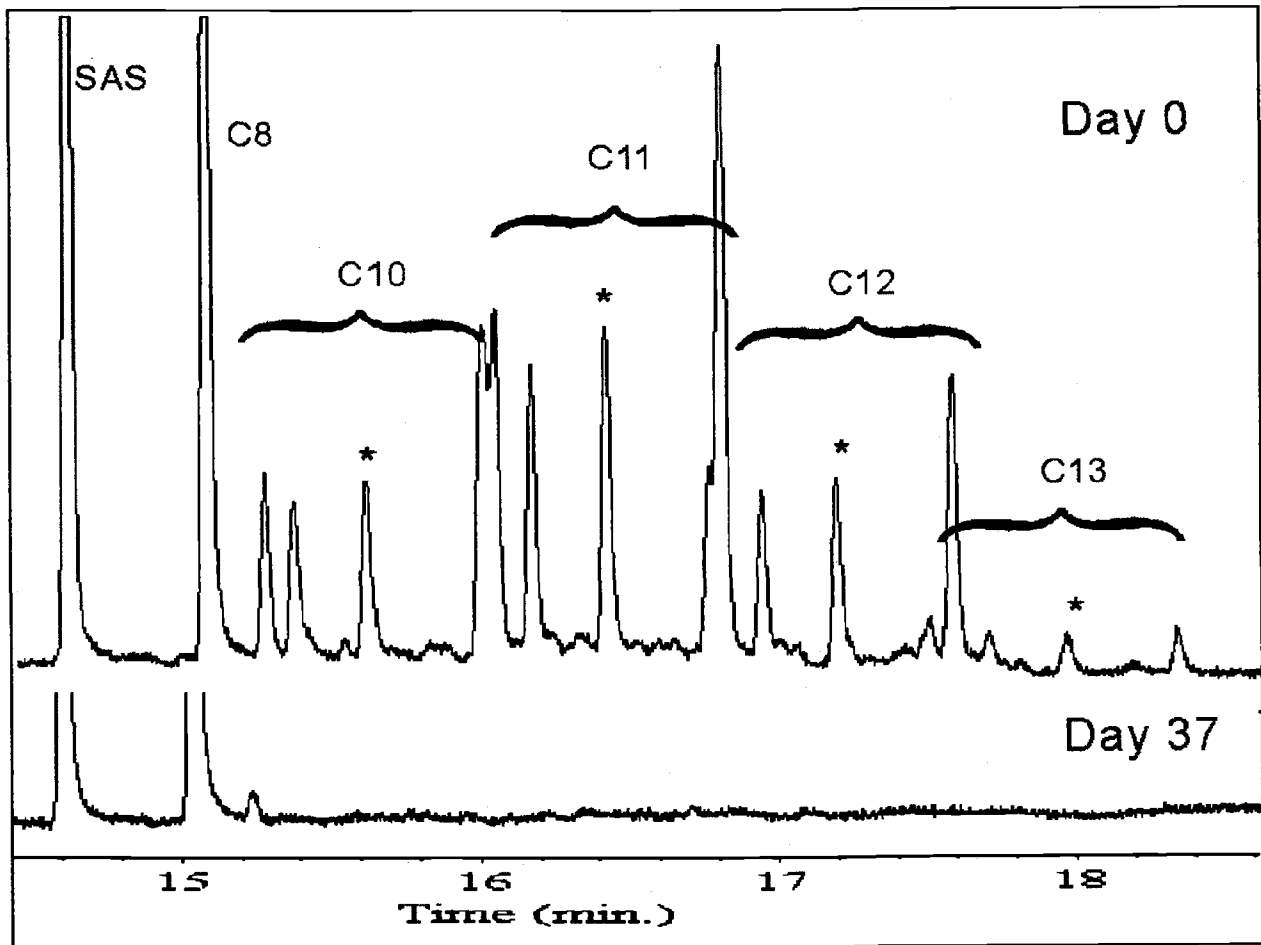


Figure 5: 25°C day 37 LAS chromatogram vs. day 0 LAS chromatogram.

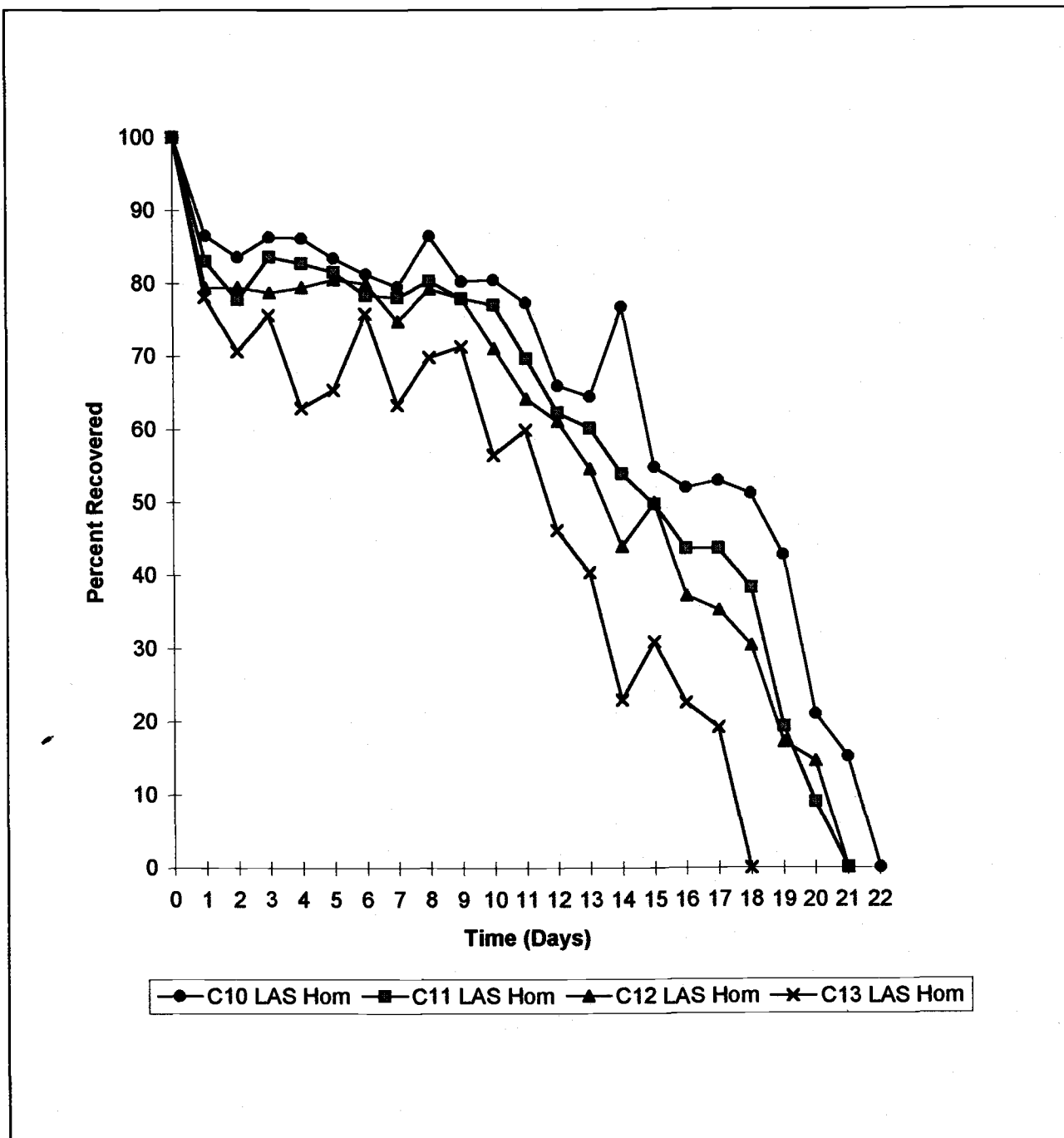


Figure 6: Percent LAS homologue recovery at 25°C.

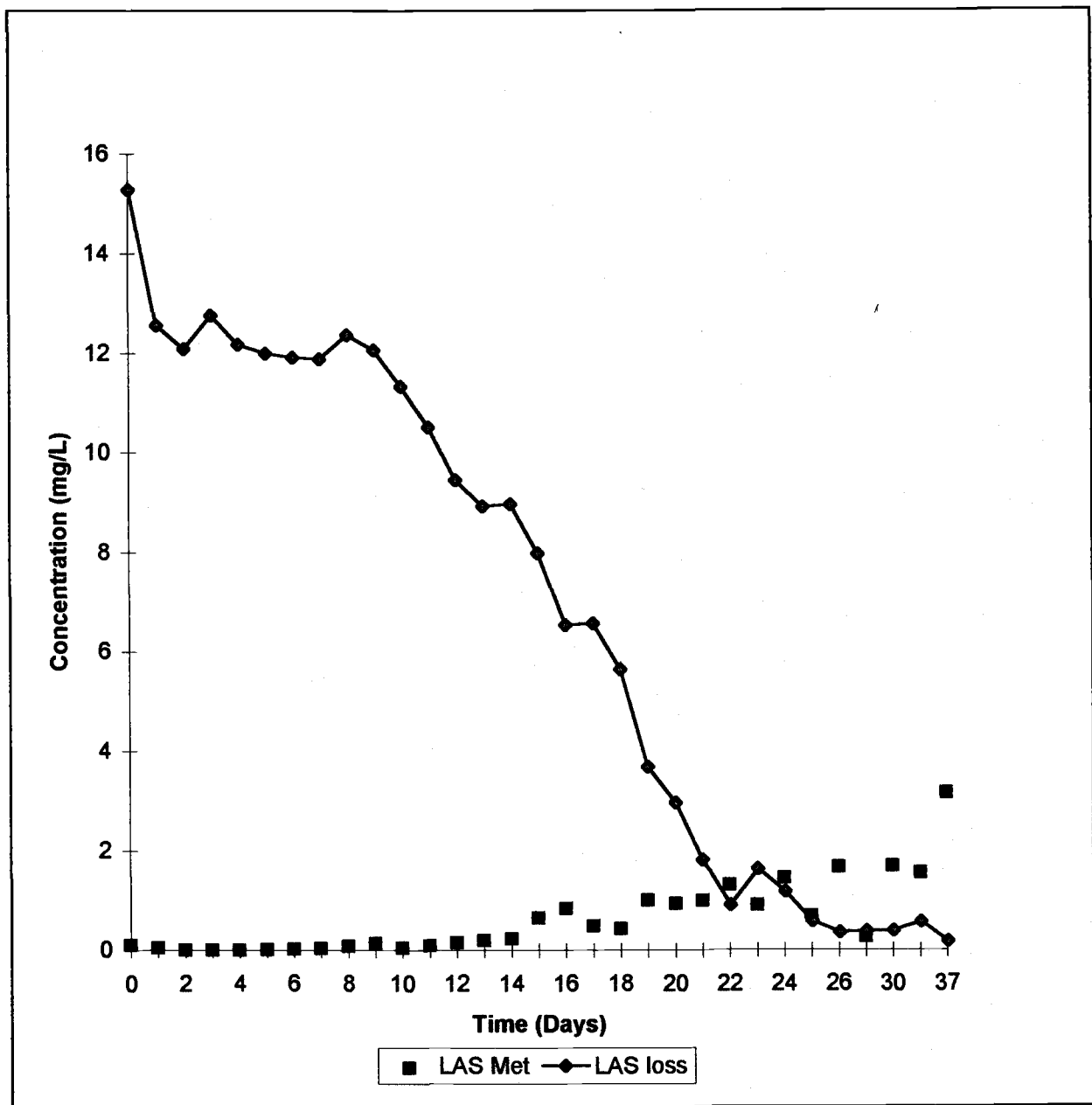


Figure 7: LAS metabolite formation at 25°C.

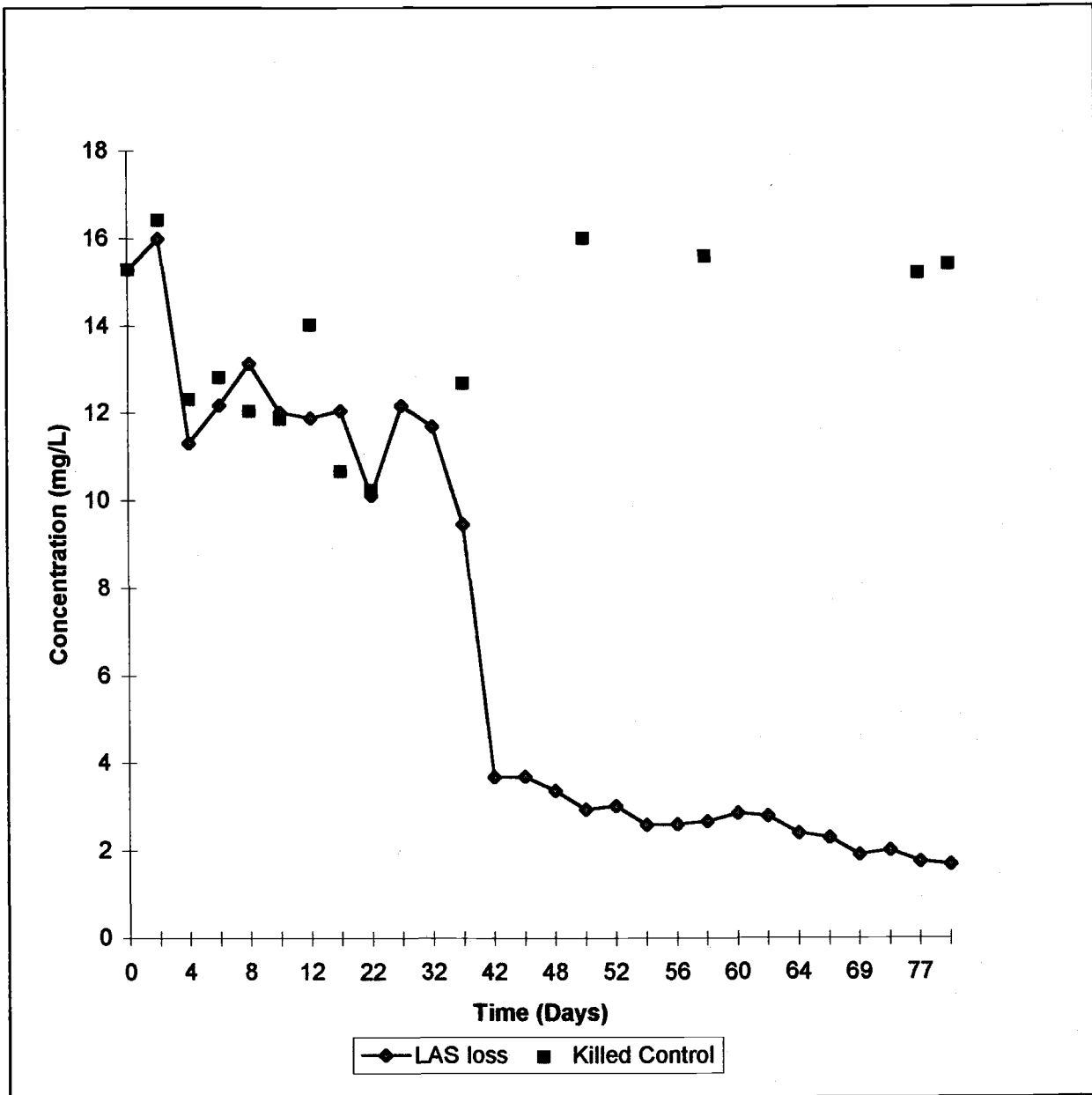


Figure 8: LAS loss at 10°C.

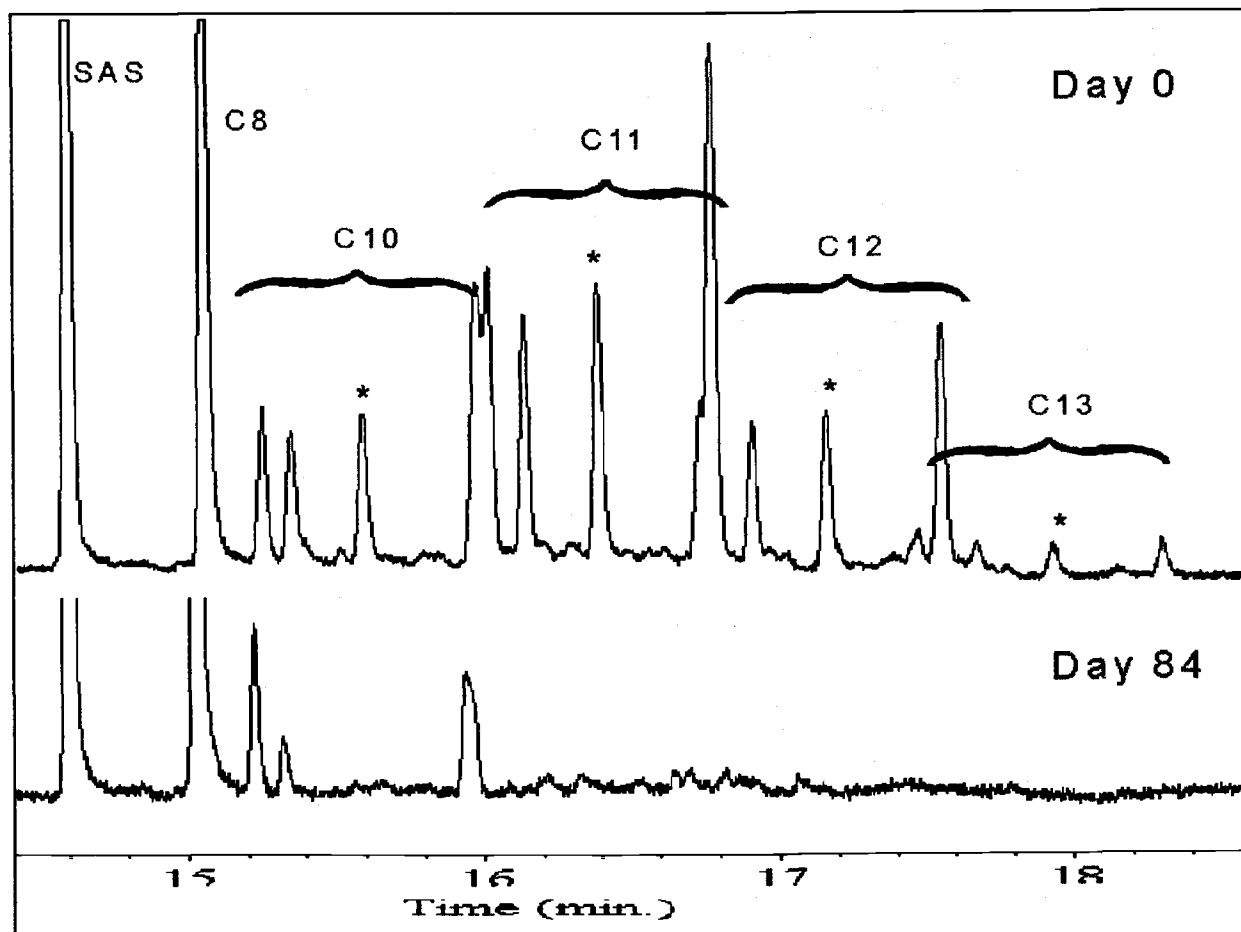


Figure 9: 10°C day 84 LAS chromatogram vs. day 0 LAS chromatogram.

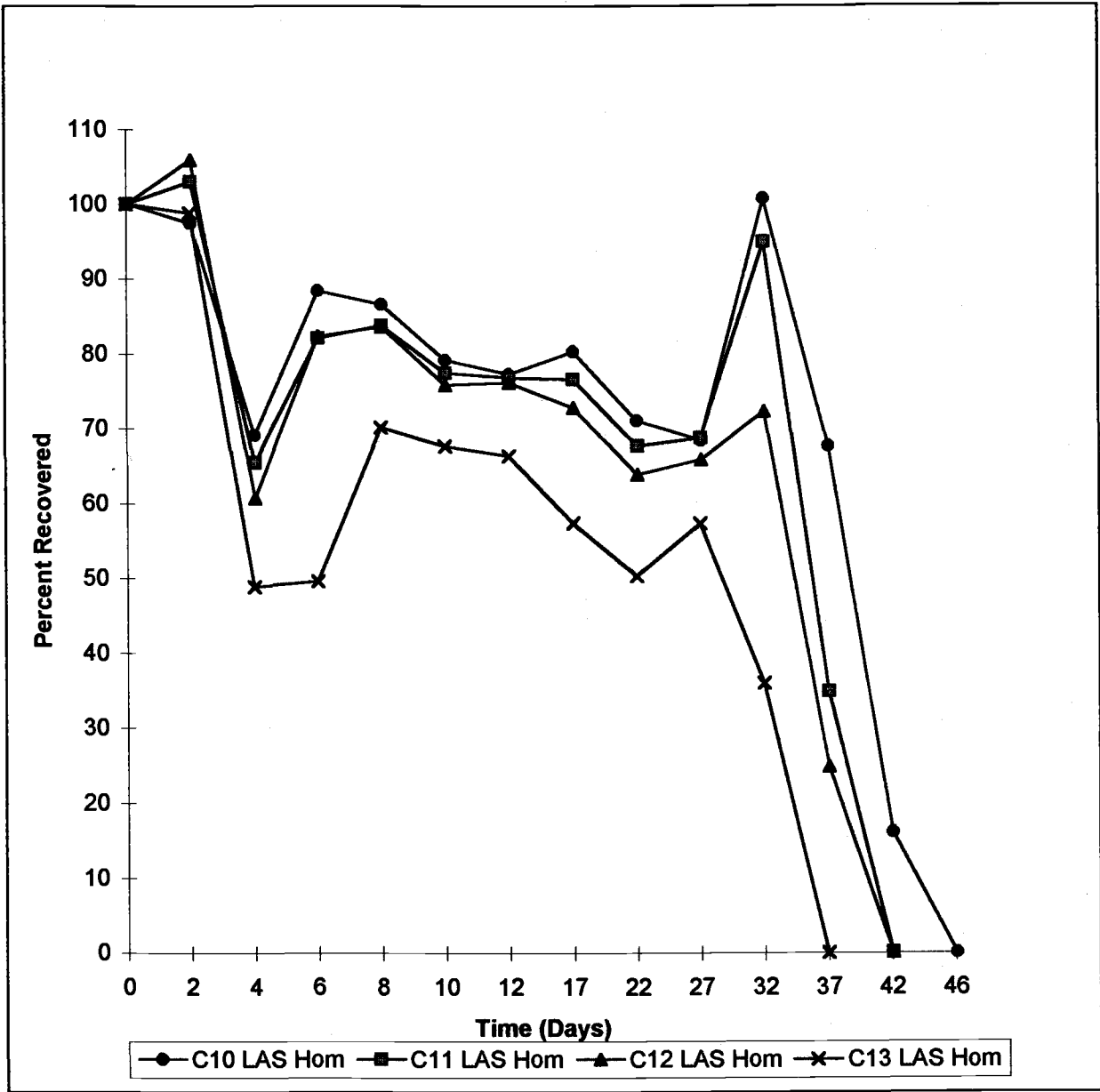


Figure 10: Percent LAS homologue recovery at 10°C.

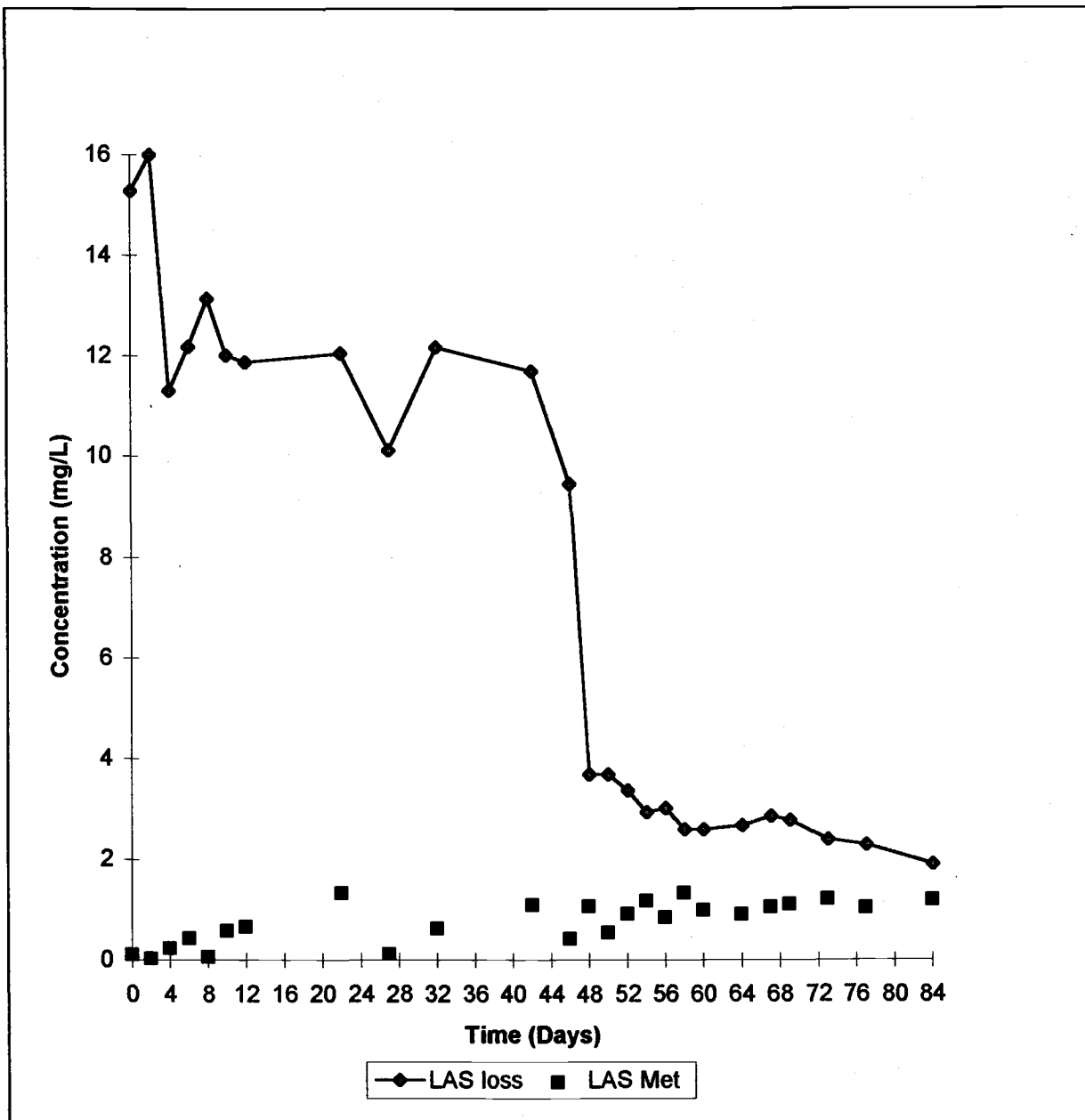


Figure 11: LAS metabolite formation at 10°C.