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Determination of anionic surfactants during wastewater recycling process by ion pair chromatography with suppressed conductivity detection

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Abstract

A direct approach utilizing ion pairing reversed-phase chromatography coupled with suppressed conductivity detection was developed to monitor biodegradation of anionic surfactants during wastewater recycling through hydroponic plant growth systems and fixed-film bioreactors. Samples of hydroponic nutrient solution and bioreactor effluent with high concentrations (up to 120 mS electrical conductance) of inorganic ions can be analyzed without pretreatment or interference. The presence of non-ionic surfactants did not significantly affect the analysis. Dynamic linear ranges for tested surfactants [Igepon TC-42, ammonium lauryl sulfate, sodium laureth sulfate and sodium alkyl (C_{10}–C_{16}) ether sulfate] were 2~500, 1~500, 2.5~550 and 3.0~630 μg/ml, respectively. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Anionic surfactants; Wastewater recycling

1. Introduction

Surfactants are the active ingredients in household detergents, industrial cleaning agents and personal care products. Surfactants used in personal care preparations account for an estimated 15–16% of the total surfactant consumption for the time period between 1977 to 1992 [1]. Among the different types of surfactants, anionic surfactants are the most widely used as primary surfactants in personal care products. They are almost exclusively organic sulfonates and sulfates with some being carboxylates such as bar soaps. Accurate measurement of these surfactants in wastewater or environmental samples has always been a challenge due to their foaming and amphiphilic nature and general lack of light absorbance.

Methylene blue active substances (MBASs) have long been used as a standard method for measuring sulfonate and sulfate-based anionics in wastewater [2]. While this method is sensitive, with a detection limit of 10 μg in a 100-ml sample, it is time-consuming and often interfered with by sample matrix. Substances such as organic sulfonates, sulfates, carboxylates and phenols and inorganic thiocyanates, cyanates, nitrates and chlorides may transfer more or less methylene blue into the chloroform phase and result in positive interference. Negative interference can result from the presence of cationic surfactants and other cationic materials (e.g., amines), because they compete with the methylene

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blue in the formation of ion pairs. Particulate matter may give negative interference through adsorption of MBASs. The method also requires a large amount of sample (100 ml) and uses a large quantity (more than a 100 ml/sample) of the toxic solvent, chloroform.

There are several direct analytical approaches based on titration of ionic surfactants with surfactants of an opposite charge [3]. Potentiometric titration with surfactants of opposite charge using a surfactant-sensitive electrode was applied to analysis of Igepon by Wisniewski and Bubenheim [4], but proved to be cumbersome and poorly reproducible. Other more specific and sophisticated methods using gas chromatography–mass spectrometry (GC–MS) [5–7] require intensive sample preparation and expensive equipment. Although high-performance liquid chromatography (HPLC) coupled with fluorometric detection was successfully used in monitoring of aromatic surfactants in sewage influent and effluent upon solid-phase extraction [8], pre-column derivatization is normally required for non-chromophoric surfactants [9].

Separation and detection of anionic surfactants by mobile-phase ion chromatography and suppressed conductivity was attempted by Weiss [10]. However, isocratic elution in his work did not effectively separate different components in some technical products, and no quantitative analysis was conducted. The development of highly efficient packing material (e.g., crosslinked, macroporous copolymer of polystyrene and divinylbenzene) has greatly improved the separation efficiency of high-molecular-mass ionic compounds. Incorporation of a flat-sheet membrane suppressor not only lowers the detection limits, but also allows the use of high-capacity columns and gradient elution [11] that further enhances separation. This paper presents a direct and rapid method for analysis of sulfonated and sulfated anionic surfactants, as well as results from the application of this method for monitoring Igepon TC-42 degradation in two different wastewater processes.

2. Experimental

2.1. Reagents

Acetonitrile (HPLC grade), sodium lauryl sulfate and polyoxyethylene 10 lauryl ether were obtained from Sigma (St. Louis, MO, USA). ACS certified ammonium hydroxide (14.8 *M*, Fisher Scientific, Pittsburgh, PA, USA) was used as an ion pair reagent. Mobile phase and control samples were made from deionized, organic-free water (>18 MΩ). Whole body shampoo/hand cleaner containing 24% active ingredient, Igepon TC-42 (Ecolabs, St. Paul, MN, USA) was used to make up simulated graywater for wastewater process tests through the hydroponic plant growth system and bioreactor. Rhodapon L-22, Rhodapex ES2 and Rhodapex ESY that contain 27% ammonium lauryl sulfate, 25.1% sodium laureth sulfate and 25.03% sodium C_{10}–C_{16} ether sulfate, respectively, were courteously provided as samples by Rhodia (Rhodia Surfactants and Specialties Group, Cranbury, NJ, USA). Chemical structures of surfactants tested in this study are illustrated in Fig. 1.

2.2. Instrumentation and analysis

A Dionex DX-500 system (Dionex, Sunnyvale, CA, USA) equipped with a conductivity cell (DS3) and self-regenerating suppressor (Dionex ASRS Ultra 4 mm) was used. Separation was achieved on a Dionex IonPac NS1 column (10 μm, 250 mm×4 mm) using a gradient of acetonitrile and 5 m*M* ammonium hydroxide. The following gradient program was employed throughout the experiment unless stated otherwise: 5% acetonitrile for first 5 min followed by a linear increase to 45% over the next 15 min and a hold at 45% for 5 min. Flow-rate of the mobile phase was kept at 1 ml/min. Filtered samples (100 μl) were introduced to column by an autosampler (AS3500 autosampler). The suppressor was operated in the external chemical mode using 5 m*M* sulfuric acid as a regenerant.

2.3. Determination of Igepon concentration in hydroponic media supporting wheat growth and in bioreactor effluent

Samples with Igepon concentrations greater than 2 μg/ml, but less than or equal to 500 μg/ml were directly filtered through syringe filters with 0.2-μm membranes into autosampler vials. Otherwise, samples were concentrated or diluted accordingly. Sam-

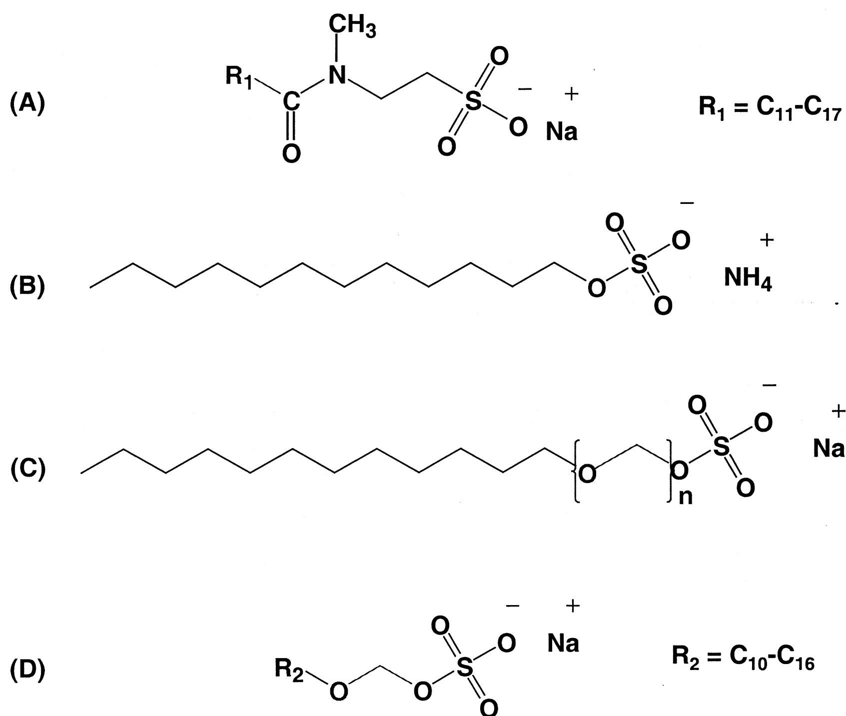

Fig. 1. Chemical structures of surfactants tested in this study. (A) Igepon TC-42, (B) ammonium lauryl sulfate, (C) sodium laureth sulfate, (D) sodium alkyl (C_{10}–C_{16}) ether sulfate.

ples were analyzed immediately or stored in a −25°C freezer.

Wheat (*Triticum aestivum* L. cv. Apogee) was grown in recirculating hydroponic system containing 1/2 strength Hoagland's solution [12]. Simulated graywater containing 100, 300 and 900 μg/ml of Igepon was added to separate 20-L reservoirs to compensate for water loss due to evapo–transpiration of the plants. On day 34 after planting, all three reservoirs were dosed with 2.2 L graywater stock solution containing 900 μg/ml of the surfactant. Samples were taken at different time points after this dosing in order to evaluate the wastewater processing capability of the three different systems that were previously exposed to different levels of surfactant.

Igepon concentration was monitored in a second system under evaluation for graywater bioprocessing, a fixed-film bioreactor. The bioreactor design consisted of a cylindrical reactor (245 mm×77 mm, H×D), packed with porous ceramic beads for biofilm formation, and an adjoining liquid–gas separator with a total system volume of 1250 ml. The feed and effluent were stored in a chilled water bath maintained at 2–4°C to prevent microbial biodegradation outside of the reactor. Feed material containing Igepon surfactant at a concentration between 400 and 700 μg/ml was added at the same rate that effluent was removed (0.6 ml/min). A retention time of the effluent of 36 h was maintained throughout the experiment. A microbial inoculum originating from the rhizosphere of wheat plants grown in the hydroponic system at Kennedy Space Center was cultured in a nutrient rich medium [0.8 m*M* $MgSO_4$, 8.3 m*M* $(NH_4)_2SO_4$, 2.6 m*M* K_2HPO_4, 49 m*M* KNO_3] prior to being added to the reactor in a 10% (v/v) ratio. The experiment sought to evaluate the feed composition in order to determine necessary nutrient composition for optimal microbial biodegradation capability. Graywater feed stream contained a single nutrient KNO_3 (49 m*M*), for the first 8 days of the experiment, but was restored to a full spectrum of nutrients used in the culturing of microbial

inoculum from day 9 onward. Surfactant concentration was determined for both effluent and feed with samplings at 24-h intervals.

3. Results and discussion

3.1. Ion chromatography of Igepon TC-42 in Ecolab cleanser

Ammonium hydroxide and tetrabutyl ammonium (TBAOH) hydroxide were tested as ion pairing reagents. The former results in shorter retention time and better separation between the constituent components of Igepon. TBAOH is a preferred ion pairing reagent for analysis of the ionic analytes with low molecular masses because it is compatible with organic solvents and enhances hydrophobic interaction between the analytes and column packing material. However, since surfactants tested consist of a hydrocarbon chain of more than 12 carbons, the ion pairing with TBAOH adds an additional 16 carbons, resulting in increased hydrophobicity and interaction between hydrophobic column packing material and ion paired molecules. Therefore, ammonium hydroxide was chosen over TBAOH in this application. A concentration of 5 m*M* ammonium hydroxide was sufficient to achieve the desired result. Although isocratic elution using 30% acetonitrile resulted in complete separation of all the components in Igepon within 20 min, a gradient elution (see Experimental section) was required in order to eliminate the interference of inorganic ions (indicated by arrow, Fig. 2) present in the samples. Regardless of sample matrices, baseline separation of all individual components in Igepon TC-42 was achieved (Fig. 2). The large quantity of inorganic ions and other interference present in plant hydroponic medium and bioreactor medium did not affect the separation efficiency, e.g., the resolution $[R_s=2(t_{r2}-t_{r1})/(T_{w1}+T_{w2})]$ for first pair peaks remained 3.6 in all three media tested.

Peaks 7, 8, 9, 10 and 11 in Fig. 2 were assigned to *N*-methyltaurates of lauric, myristic, palmitic, oleic and stearic acids, respectively based on the percentage of fatty acid components determined by GC–MS [5] and the assumption that all these molecular species have the same equivalent conductance. The detector responses of these components in matrices of increased ion strength were presented as percentage of the response in control (Table 1). Inorganic ions differentially affect the recovery of components with varied chain lengths and saturation. The recovery of *N*-methyltaurate of lauric acid in these matrices was not significantly changed (the difference is less than 1%), while that of myristic acid increased and that of the other three components decreased when the interference presented in sample matrix. The decrease in response became more profound as concentration of Hoagland's solution and the alkyl chain length increases. The cause for the increased recovery of myristic N-methyltaurate is unclear since analysis of blank samples (Hoagland's solution without surfactant) did not give any indication of the presence of coeluting compounds. The most likely cause for the decreased recovery of the longer alkyl chain components is their irreversible binding to the double valent ions (2.5 m*M* Ca^{2+} and 1.0 m*M* Mg^{2+} in 1/2 strength Hoagland's). This interpretation is based on: (1) our observation that addition of sodium chloride (up to 2.8 m*M*, 340 μS) resulted in only slight decrease in the recovery of the stearic component (data not shown); (2) the known fact that water containing Ca^{2+} and Mg^{2+} (hard water) consumes more detergent than soft water due to the partial loss of surfactants; and (3) previous observation of increased adsorption of surfactant molecules to the inorganic matter in sample matrix as alkyl chain length increases [8]. The observed interference did not significantly limit the application of the method for quantification of total Igepon TC-42, since the major component (lauryl *N*-methyltaurate) used for quantification was negligibly affected.

The method has a dynamic linear range between 2 and 500 μg/ml (Amt$=3.44\cdot10^{-5}\times$peak area$+1.69$). An eight-level calibration (two injections per concentration level) has correlation coefficient of 0.9999. Under the experimental conditions used, the minimum quantification limit for Igepon TC-42 is 0.2 μg of total surfactant on the column. The flow-rate of external regenerant is critical in order to ensure reproducibility and the detection limit. A flow-rate of 4 ml/min was maintained in this study by pressure in the headspace of a reservoir bottle.

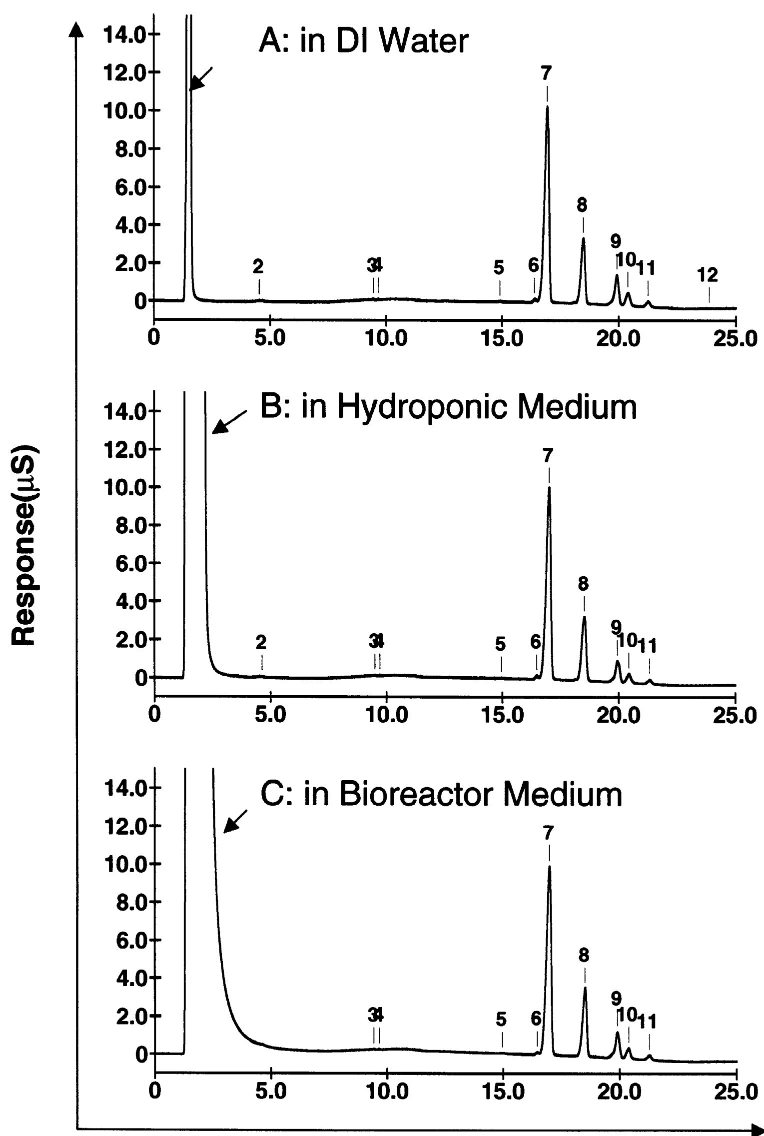

Fig. 2. Effect of sample matrix on the separation and detector responses of surfactant components. (A) 54.1 μg/ml Igepon in DI water, (B) 54.1 μg/ml Igepon in hydroponinc medium and (C) 54.1 μg/ml Igepon in bioreactor medium.

Table 1
Effect of inorganic ions on quantification of Igepon constituents; data reported as percent change in response compared to deionized control

Matrix	EC of matrix (μS)	Lauric	Myristic	Palmtic	Oleic	Stearic
1/8 Strength Hoagland's	53	0.0	6.0	−3.0	−16.6	−35.2
1/4 Strength Hoagland's	105	0.2	9.3	−8.6	−23.1	−34.1
1/2 Strength Hoagland's	198	0.6	12.1	−15.3	−28.9	−29.0

3.2. Application to determination of other anionic surfactants

The method is equally effective for determination of the common active components in most commercial liquid personal care products (ammonium lauryl sulfate, sodium laureth sulfate, and sodium C_{10}–C_{16} ether sulfate). The same conditions described in the Experimental section were sufficient to separate surfactant molecules in Rhodapon L-22, while a shallower gradient (5% acetonitrile for first 5 min followed by a linear increase to 35% over next 15 min and a hold at 35% for 8 min) was required to better separate the constituent surfactant molecules in Rhodapex ES2 and Rhodapex ESY (Fig. 3). Four molecular species were detected in Rhodapon L-22. The peak labeled as ALS in Fig. 3A co-eluted with a pure form of sodium lauryl sulfate; it was subsequently identified as ammonium lauryl sulfate and used for quantification. Similarly, the well separated main peaks (indicated by an arrow) in Fig. 3B and C served as quantification basis. The peak area of this component has a linear relationship with the total surfactant concentration in the ranges shown in Table 2.

It is not possible to quantify individual surfactants in a sample containing a mixture of the surfactants mentioned above because molecular species with similar or the same carbon chain length occur in Igepon, sodium laureth sulfate and sodium alky (C_{10}–C_{16}) ether sulfate. The total concentration of anionic surfactants can be easily determined even in the samples containing non-ionic surfactants due to the selectivity of suppressed conductivity detection. Detector response of ammonium lauryl sulfate (86.6 μg/ml) was increased by less than 3% in the presence of polyoxyethylene 10 lauryl ether (a non-ionic surfactant) at levels as high as 300 μg/ml (Table 3). This slight increase in the response may be due to the decreased Igepon loss resulting from adsorption to sample vials and other surfaces as the concentration of co-existing non-ionic surfactant increases.

3.3. Application to monitoring of Igepon concentration during wastewater processes

In an effort to recycle graywater into potable water for NASA's advanced life support project, the biodegradabilty of Igepon TC-42 in a whole body shampoo from Ecolabs was tested through two different approaches. One was the direct recycling of graywater through a hydroponic plant growth system, where microorganisms associated with plant roots biodegrade the surfactant. An alternate method for graywater recycling was through the use of a fixed-film bioreactor. The method described above was used to monitor the biodegradation or accumulation of the surfactant in the hydroponic system and bioreactor. Surfactant degradation was more rapid in treatments exposed to chronically higher Igepon concentration during the plant experiment (Fig. 4). These data agree with the observed increase in microbial activity as well as the degrader density in rhizospheres exposed to higher concentrations of Igepon (unpublished data).

Igepon concentration in both influent and effluent of the bioreactor was monitored during a span of 21 days from the initiation of the experiment (Fig. 5). Igepon concentration was below the detection limit for the first 5 days, despite the lack of nutrients in the feed. These data suggest that the inoculum provided sufficient nutrients for maximal microbial activity. After day five, however, Igepon concentration in the effluent started to increase even though the rate of Igepon addition was constant, indicating sub-optimal performance of the bioreactor. A trend of increasing surfactant concentration continued until day nine when a full spectrum of nutrients was added to the feed stream, resulting in a rapid decrease in effluent Igepon concentration. The Igepon concentration stayed below the detection limit for the remainder of the study. The method of analysis employed for surfactant degradation allowed for rapid recognition of a problem that resulted in a corrective action early in the experiment.

4. Conclusions

The method of ion pair chromatography coupled with suppressed conductivity detection presented here has proved specific and sufficiently sensitive for quantitative determination of sulfonated and sulfated anionic surfactants. It has a dynamic linear range of over two magnitudes. No extraction or derivatization

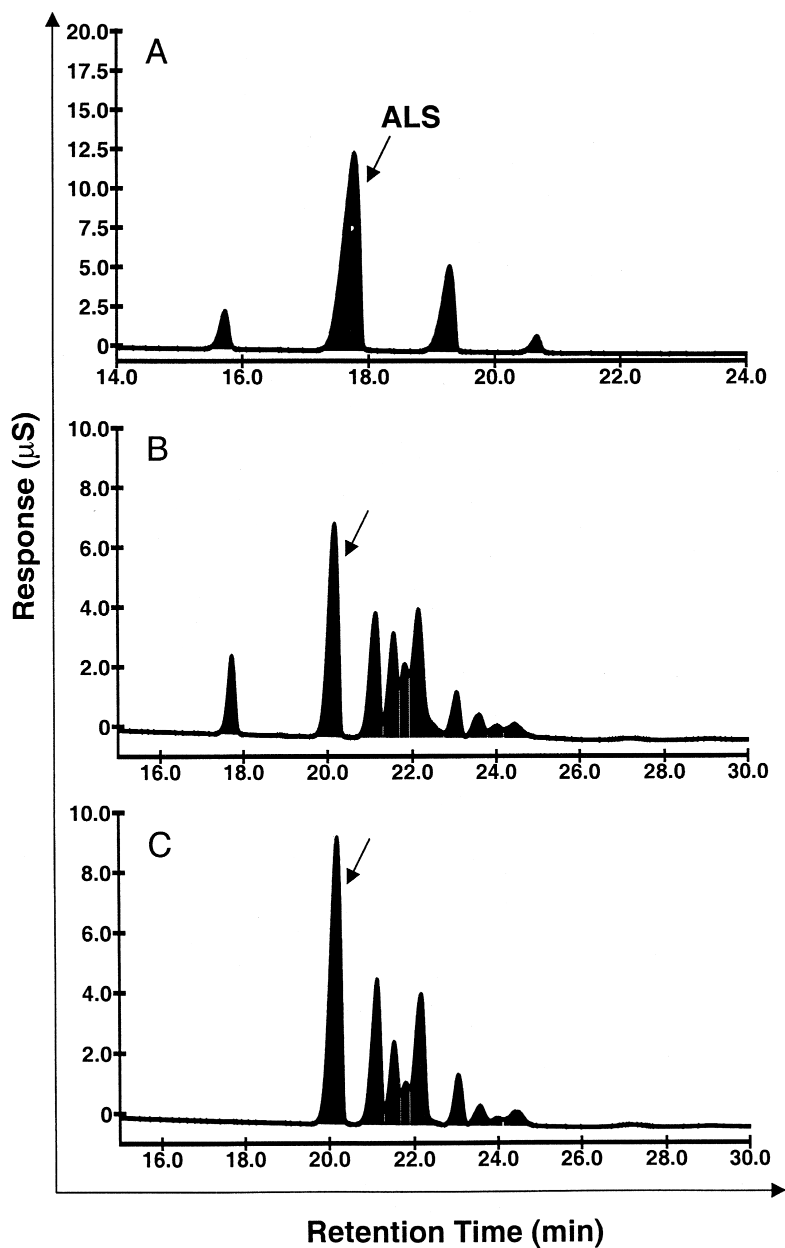

Fig. 3. Compositional fingerprints of technical products. (A) Rhodapon L-22, (B) Rhodopex ES2 and (C) Rhodapex ESY, ALS: ammonium lauryl sulfate. Peaks indicated by an arrow were used for quantification.

is needed, which makes it especially suitable for automated analysis of a large number of samples. It has been successfully applied to monitor the biodegradation of Igepon TC-42 in a hydroponic plant growth system and a fixed-film bioreactor, and will be used for monitoring the biodegradation of the other anionic surfactants as part of our continuing graywater recycling research. This method should be

Table 2
Dynamic linearity of anionic surfactant concentration and peak area

Surfactants	Linear regression	Dynamic linear range (μg/ml)
Ammonia lauryl sulfate	Conc.$=2.89\cdot10^{-5}\times$peak area-0.32 $R^2=0.9999$	1.0~500
Sodium laureth sulfate	Conc.$=9.94\cdot10^{-5}\times$peak area$+3.36$ $R^2=0.9998$	2.5~550
Sodium C_{10}–C_{16} ether sulfate	Conc.$=5.74\cdot10^{-5}\times$peak area$+3.40$ $R^2=0.9997$	3.0~630

Table 3
Effect of non-ionic surfactant on quantification of anionic surfactant

Concentration of non-ionic surfactant (μg/ml)	Response of ALS (peak area)	% Change
0.0	3 011 749	0.0
60.9	3 019 594	0.26
121.7	3 042 031	1.01
304.3	3 095 472	2.78

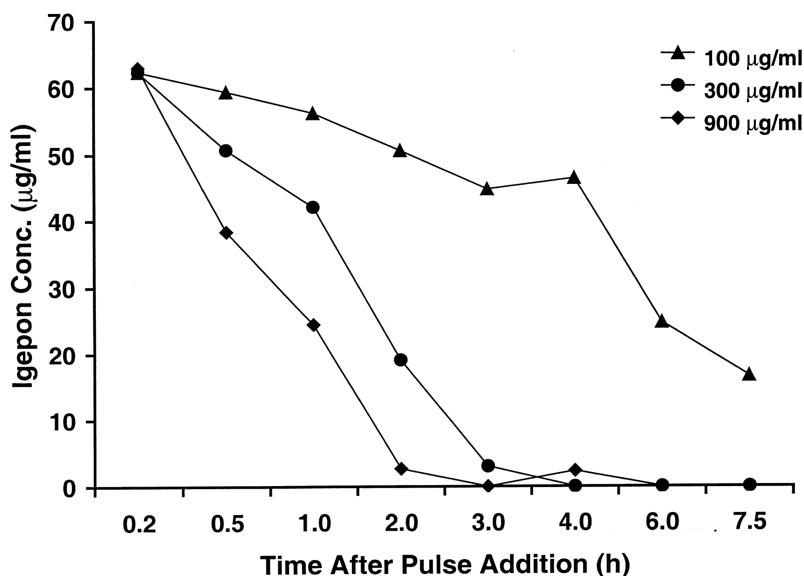

Fig. 4. Biodegradation rate of Igepon in recirculated hydroponic media previously exposed to different levels of the surfactant.

useful for other applications involving the degradation or production of anionic surfactants when intensive spatial or temporal sampling is required.

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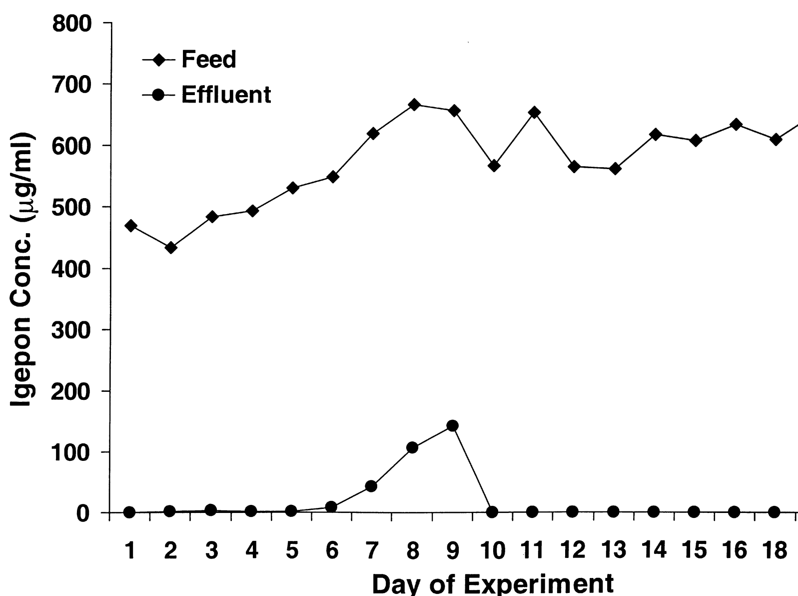

Fig. 5. Effect of nutrient composition in a fixed-film bioreactor on surfactant biodegradation.

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