Wat. Res. Vol. 24, No. 8, pp. 965–972, 1990
Printed in Great Britain.

0043-1354/90 $3.00 + 0.00

BIODEGRADATION AND SECONDARY EFFLUENT TOXICITY OF ETHOXYLATED SURFACTANTS

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(First received July 1988; accepted in revised form January 1990)

Abstract—Several aromatic- and aliphatic-based, ethoxylated surfactants were tested for their biodegradability and aquatic toxicity reduction. For all the tested surfactants, almost complete primary biodegradation by the activated sludge process was achieved, as measured by the CTAS test. Aliphatic-based products demonstrated better biodegradability in terms of TOC and COD reduction efficiency than aromatic-based products. Toxicities of individual surfactants, their mixtures and effluents from biological reactors treating surfactant mixtures were determined using *Mysidopsis bahia*. For aromatic-based (i.e. nonlyphenol-based) surfactants with the same ethylene oxide molar ratio, the toxicity of the non-biodegraded product was highest for the non-ionic (unsubstituted) surfactant. Toxicities of the non-biodegraded, aliphatic-based surfactants were in the same range as those of the non-biodegraded aromatic-based products. Increasing ethylene oxide molar ratios resulted in an exponential decrease in surfactant toxicities.

Biological treatment of the aliphatic-based surfactants resulted in non-toxic effluents even at high (600 mg/l) influent concentrations. Effluents from reactors treating aromatic-based surfactants demonstrated markedly higher toxicities than those from treatment of aliphatic-based products. The presence of an active group appeared to have less effect on biological effluent toxicity than did the product base structure (i.e. aromatic or aliphatic).

Key words—surfactant biodegradation, ethoxylated surfactants, aquatic toxicity, *Mysidopsis bahia*

INTRODUCTION

Ethoxylated surfactants constitute a major group of synthetic surface active agents, with about 15% of the U.S. surfactant market. Total U.S. production of non-ionic alkylphenol-based (mostly nonylphenol) and alcohol-based ethoxylated surfactants reached about 800 million pounds in 1985 (Broxterman and Dean, 1986). Relatively minor quantities of anionic, mostly sulfonated and phosphated, ethoxylated surfactants are produced for specialty markets. Due to the large production volume and increasing environmental concerns, a substantial research effort was devoted to surfactants biodegradation. This research effort was initially directed toward primary biodegradation of surfactants, as measured by reduction in foaming capability, surface tension recovery and responses to surfactant-specific analyses such as methylene blue active substances (MBAS) and cobalt thiocyanate active substances (CTAS). As a result, new, environmentally safer products were developed. In recent years, however, emphasis has shifted from primary biodegradation to the ultimate fate and impact of the recalcitrant byproducts of surfactant biodegradation on the environment. This change in emphasis has undoubtedly been fostered, at least in the U.S., by the growing use of aquatic toxicity testing requirements in wastewater discharge permits.

The objective of the study reported herein was to compare biodegradation and toxicity reduction of selected ethoxylated surfactants in parallel, continuous-flow activated sludge reactors at very high (several hundred mg/l) concentrations such as those found in process wastewaters from surfactant manufacturing facilities. The effect of active group substitution at the end of the ethylene oxide chain and the effect of the base structure type (aliphatic or aromatic) were of particular interest.

BIODEGRADATION PATHWAYS AND TOXICITIES OF ETHOXYLATED SURFACTANTS

Primary biodegradation of alcohol-based ethoxylated surfactants (AE), as measured by CTAS, was found to be almost complete under both laboratory conditions (Conway and Waggy, 1966; Patterson *et al.*, 1967; Stiff *et al.*, 1973) and field conditions (Conway and Waggy, 1966; Sykes and Rubin, 1979). Primary biodegradation (again, as measured by CTAS) of branched, nonylphenol-based ethoxylated surfactants, varied from 80% (Conway and Waggy, 1966) to almost complete (Lashen *et al.*, 1966; Kravetz *et al.*, 1983). The extent of ultimate biodegradation (i.e. mineralization) was highly dependent on the environmental conditions and varied from a few percent to more than 90% (Lashen *et al.*, 1966; Hartman *et al.*, 1967; Pitter and Fuka, 1979; Fuka and Pitter, 1980; Gerike and Jasiak, 1984).

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Where AE, which have predominantly linear alkyl chains, have been compared directly with alkylphenol-based ethoxylated surfactants (APE), primary biodegradation of AE proceeded considerably faster than that of APE (Mann and Reid, 1971; Stiff *et al.*, 1973; Kravetz *et al.*, 1983). Stiff *et al.* (1973) reported that, while AE primary degradation was not temperature-dependent in the 8–15°C range, APE biodegradation at an influent concentration of 20 mg/l was slower and unstable at lower temperatures. Similarly, APE treatment sensitivity to low temperatures was noted by Mann and Reid (1971) and Painter and King (1978).

The initial step in the biodegradation of ethoxylated non-ionic surfactants could involve bacterial attack at the far end of either the hydrophobe or the ethylene oxide (EO) chain, or it could involve a central fission separating the hydrophobe and the EO chain (Swisher, 1987). For AE, all of these pathways are considered important. For APE, however, attack at the terminal EO group resulting in shortening the EO chain by one unit at a time (ω–EO pathway) has been documented most often (Swisher, 1987).

Three types of metabolic byproducts of tripropylene derived nonylphenol ethoxylates (tp-NPE) biodegradation were isolated by different workers. Most frequently, exthoxamers with a low number of EO units (low ethoxamers), were identified, consistent with the ω–EO biodegradation pathway (Osburn and Benedict, 1966; Rudling and Solyom, 1974; Kravetz *et al.*, 1983; Ahel and Giger, 1985), indicating a piece-wise shortening of the EO chain. Osburn and Benedict (1966) found evidence of the presence of a carboxyl group on the hydrophobe, which would indicate that oxidation of the highly branched carbon-chain is possible. Schobert *et al.* (1981) reported that only hydrophobe carboxylates of NPE_2 were present in the activated sludge effluent, rather than unoxidized NPE_2. Bruschweiler *et al.* (1983) concluded that a major component of the NPE_7 metabolic matrix is NPE_2 with its terminal EO group oxidized to form carboxymethyl NPE_1. Recently, Yoshimura (1986) confirmed the formation of NP acetates and ethoxyacetates. All of the proposed biodegradation pathways with their respective byproducts are most likely possible. Their relative importance depends on the environmental conditions under which the biodegradation occurs.

Toxicities of ethoxylated surfactants decrease as their EO molar ratios increase, as demonstrated by Yoshimura's (1986) data for a series of nonylphenol ethoxamers (48 h LC_{50} on Japanese Killifish, *Oryzias latipse*). Toxicities of NPE_0 and NPE_1 carboxymethyls corresponded to the toxicities of much longer unoxidized ethoxamers (7–8), as can be expected from a comparison of their hydrophilicities. Consequently, the toxicity of an APE_n surfactant can either decrease or increase upon biodegradation, depending on the metabolic pathway.

MATERIALS AND METHODS

Surfactants

Surfactants selected for the study and their characteristics are presented in Table 1. The first three surfactants are all based on tp-nonylphenol with an ethylene oxide molar ratio of nine. Each product has a different active group at the end of the ethoxy chain. Product A is an unsubstituted nonylphenol ethoxylate (NPE_9). In products B and C, the terminal hydroxyl group of NPE_9 is substituted with OSO_3^- and OPO_3^{-2} active groups, respectively. Product D is a chloride-capped surfactant with an aliphatic base (tridecyl alcohol). Products E, F, G and H are aliphatic-based surfactants with various ethylene oxide molar ratios and different aliphatic base structures. All surfactants were samples of commercially available products.

Reactors operation

The surfactants were treated in five, parallel, continuous-flow, bench-scale reactors with baffled clarification sections. The reactors were seeded with activated sludge from an industrial wastewater treatment plant treating ethoxylated surfactants (among others). The reactors were operated for 10 weeks, except reactor No. 4 of which operation was terminated after 8 weeks.

Operating parameters for the reactors are listed in Table 2. Reactor No. 5 served as control with feed consisting of a mixture of products E, F, G and H. All these surfactants were aliphatic-based and were considered to have low toxicity potential following biological treatment (Maki *et al.*, 1979; Turner *et al.*, 1985). Feed to the remaining four reactors consisted of about 50% of the control reactor feed (cosubstrate), supplemented with the individual surfactants A, B, C and D. The composition of all feeds is detailed in Table 3. Feed to the reactors was prepared every other day from feed concentrates diluted with tap water and was supplemented with nitrogen, phosphorus and bicarbonate.

Table 1. Characteristics of tested surfactants*

Code No.	Surfactant†	Base type	EO molar ratio	Active group substitution	ThOD (g O_2/g)	48 h Mysid toxicity LC_{50} (mg/l)‡
A	tp-NPE_9	Nonylphenol	9	OH	2.23	1.23§
B	tp-$NPE_9OSO_3^-$	Nonylphenol	9	OSO_3H	1.93	29.6
C	tp-$NPE_9OPO_3^{-2}$	Nonylphenol	9	$OPO_3(NH_4)_2$	1.98	4.6§
D	$TDAE_{10}$Cl-capped	Tridecyl alcohol	10	Cl	2.52	0.71
E	Methyl oleoyl taurate	Oleoyl	1	SO_3Na	2.39	12.8
F	$TDAE_{9.75}$	Tridecyl alcohol	9.75	OH	2.56	2.2
G	DAE_4	Decyl alcohol	4	OH	1.97	5.6
H	Castor oil E_{30}	Castor oil	30	OH	3.01	116

*On dry organic mass basis.
†E, ethylene oxide; NPE, nonylphenol ethoxylate; TDAE, tridecyl alcohol ethoxylate; DAE, decyl alcohol ethoxylate; tp, derived from tripropylene.
‡Values determined by authors during this investigation. Values are based on a single analysis unless otherwise indicated.
§Harmonic mean of results obtained.

Table 2. Operating parameters for the reactors*

Parameter	Value or range
Reactor volume (liters)	20
Feed flow rate (l/day)	6.15
Hydraulic retention time (days)	3.25
Design feed TOC (mg/l)	500
Basin dissolved oxygen (mg/l)	2–3
Basin temperature (°C)	23–25
Basin pH	7–8
MLSS/MLVSS (mg/l)	2920/2440†

*Operating parameters were identical for all the reactors, except as noted.

†Due to excessive solids loss in the effluent, reactor No. 1 average MLSS/MLVSS was 2280/1820 mg/l.

Analytical methods

Analytical results reported in this study were obtained according to procedures specified in *Standard Methods* (APHA, 1985), with the following exceptions. The cobalt thiocyanate active substances (CTAS) analytical procedure for the determination of non-ionic surfactants (APHA, 1985) was modified by eliminating the ion exchange step. This modification resulted in all polyethoxylated surfactants being included in the CTAS measurement, thereby allowing determination of anionic surfactants. For preparation of a standard calibration curve, surfactant A (NPE_9) was used. This surfactant gave a spectrophotometric response about twice that of the EPA-provided reference surfactant (EPA No. 387).

The effluent samples for 5-day biochemical oxygen demand (BOD_5) analysis were filtered through Whatman 934-AH fiberglass filters (1.5 μm nominal pore size). This type of filter allows passage of part of the dispersed bacteria, eliminating the need for additional seed. The influent samples for BOD_5 and ultimate BOD measurements were seeded with 10 ml of a mixture of unfiltered effluents from all five reactors.

Reactor effluent and mixed liquor samples were analyzed periodically for nonylphenol and low nonylphenol ethoxylates, which, as previously discussed, are potential byproducts of the biodegradation of higher nonylphenol ethoxylates. In the analytical procedure, a sample was separated and cleaned in a continuous steam distillation–solvent (cyclohexanol) extraction apparatus (Ahel and Giger, 1985). The extract was then analyzed by GC for nonylphenol, and its mono-, di- and tri-ethoxylates. The results reported here are the sum of detected concentrations of these constituents and are referred to as NPE_{0-3}.

Toxicities of the individual surfactants, their mixtures and reactor effluents were determined by 48 h, daily static-renewal tests with *Mysidopsis bahia* (mysids). This marine species was recently introduced as a test organism for monitoring the toxicities of wastewater treatment plant effluents and other discharges to the environment in New Jersey and Florida. Tests were performed according to the procedure in "Regulations Governing Laboratory Certification and Standards of Performance", New Jersey Administrative Code, 7:18, as amended July 1984. Toxicity results were calculated by a probit method and were reported as a concentration (for untreated products) or a dilution (for effluents) resulting in the death of 50% of the test organisms (LC_{50}).

RESULTS AND DISCUSSION

Toxicities of individual surfactants and their mixtures

Results of the toxicity tests on the individual surfactants are presented in Table 1. It is apparent that, within the nonylphenol-based surfactants, active group substitution at the end of the EO chain markedly decreased product toxicity.

The toxicity of the sulfated surfactant B was about 24-fold less than the toxicity of its unsubstituted homolog (A). This effect was most likely associated with an increased water solubility of the substituted product, which in turn decreased the product's affinity for the lipid membranes of the test organisms. In unsubstituted product A, the lack of a dissociating active group at the end of the EO chain rendered the molecule less polar and, therefore, more likely to be

Table 3. Feed composition and comparison of calculated and measured LC_{50} values for surfactant mixtures (reactor's feeds)

Feed composition*						
Reactor (feed) No.	Product code†	Concentration of feed components (mg/l)	Measured individual product LC_{50}‡ (mg/l)	Individual product or component LC_{50}§ (%)	Calculated feed LC_{50}¶ (%)	Measured feed LC_{50} (%)
1					0.28	0.29
	A	385	1.2	0.32		
	Feed 5	241		2.2‖		
2					1.7	0.78
	B	385	30	7.7		
	Feed 5	241		2.2‖		
3					0.77	0.43
	C	385	4.6	1.2		
	Feed 5	241		2.2‖		
4					0.17	0.14
	D	385	0.71	0.18		
	Feed 5	241		2.2‖		
5					1.1	0.70
	E	241	13	5.3		
	F	121	2.2	1.8		
	G	96.5	5.5	5.7		
	H	24.1	120	>100		
	Total	483				

*Feed to reactors 1–4 consisted of 241 mg/l of feed 5 and 385 mg/l of different surfactants.

†Refer to Table 1 for surfactant identification.

‡48 h toxicity to *Mysidopsis bahia*.

§At the concentration present in the feed, as calculated from formula (A1).

¶Calculated using formula (A2).

‖Feed 5 LC_{50} at a concentration of 483 mg/l used for reactor 5 is calculated at 1.1%, as presented elsewhere in this table. For preparation of feeds 1–4, feed 5 was added at a concentration of 241 mg/l, hence LC_{50} of this component was 2.2% [formula (A1)].

adsorbed on cell membranes with an associated toxic effect.

Aliphatic-based products demonstrated a wide range of toxicities, with LC_{50} values from 0.7 to 116 mg/l. The results presented in Table 1 indicate that the type of surfactant base structure (aromatic or aliphatic) did not appear to be an important factor in the pure product toxicity.

In addition to the surfactants listed in Table 1, a series of nonylphenol ethoxamers with different EO molar ratios was tested for mysid toxicity. The results are presented in Fig. 1 together with related literature data. From this figure, it is apparent that the toxicity of nonylphenol ethoxamers decreases exponentially with EO chain length.

In addition to individual surfactants, mixtures of them (i.e. reactor feeds) were also tested for mysid toxicity. Since individual surfactant LC_{50}s were determined separately (Table 1), it was possible to compare expected (calculated) and measured toxicities of the mixtures.

Formulas for determining the joint toxicity of compounds are based on a harmonic mean concept and are used in pharmacology, agriculture and pest control to quantify degree of antagonism or synergism (Marking, 1985). The application of joint toxicity formulas to LC_{50} values in wastewater treatment is illustrated in the Appendix.

The expected toxicity of each reactor's feed was calculated as the weighted, harmonic mean of individual feed components (formula A3 in the Appendix). The calculated values are compared with the experimentally determined values in Table 3. The calculated LC_{50}s were 20–100% higher than the measured values. This finding indicated the presence of a synergistic effect which limited the applicability of formula A3 (Appendix) for prediction of joint toxicity for the tested materials.

Biodegradability

The average performances of the reactors in terms of BOD_5 and total organic carbon (TOC) removals are summarized in Table 4. All the reactors achieved almost complete removal of the feeds' BOD_5. The average effluent filtered BOD_5 values of the reactors were less than or equal to 5 mg/l, except for reactor No. 4 for which the average value was 17 mg/l.

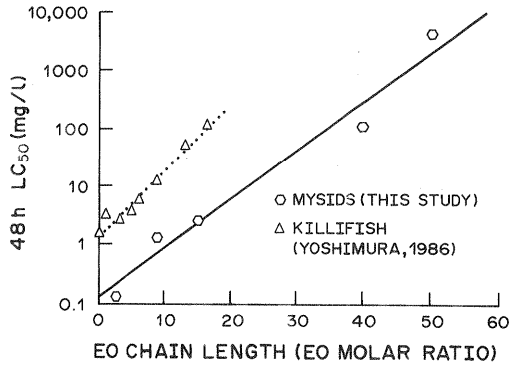

Fig. 1. Effect of ethylene oxide (EO) molar ratio on nonylphenol ethoxylate toxicity (lines indicate linear correlations using logarithms of the LC_{50} values).

Results of ultimate BOD tests (BOD_u) performed on selected reactor feeds are presented in Fig. 2. Virtually no TKN was present in the test solutions, hence the presented data reflect carbonaceous oxygen demand only. The feed to reactor No. 5 (a mixture of all aliphatic-based surfactants) approached ultimate BOD after about 20 days incubation. Based on comparison with the theoretical oxygen demand (ThOD) of the feed to No. 5, 100% biodegradation was achieved under the BOD test conditions. Approximately 35 and 25% of the ultimate BOD values of the feeds to reactor Nos 1 and 5, respectively, were exerted during the initial 5-day period of the test. For this reason, the BOD_5 removal efficiencies listed in Table 4 do not accurately represent the actual extent of the biodegradation which occurred, since the influent BOD values were apparently much greater than those indicated by the BOD_5 values.

TOC removal efficiencies for the reactors fed with aliphatic-based compounds (Nos 4 and 5) were about 90%. Reactors fed with aromatic-based products (Nos 1, 2 and 3) achieved lower TOC removal efficiencies of 70–80%.

Effluent toxicity

During the initial phase of the study, effluent LC_{50} values were generally increasing, indicating progressing acclimation. The average toxicities of the reactor effluents from the last 4 weeks of operation are presented in Table 5, along with other average influent and effluent, surfactant-specific parameters.

Table 4. Average biotreatability performance of activated sludge reactors fed with different surfactants*

Reactor No.	Influent concentration† (mg/l)			Effluent concentration‡ (mg/l)			Removal efficiency§ (%)		
	BOD_5	COD	TOC	BOD_5	COD	TOC	BOD_5	COD	TOC
1	552	1590	429	5	364	120	98.8	74.5	70.6
2	339	1540	404	4	324	91	98.5	78.0	76.6
3	251	1460	410	4	327	86	98.6	79.6	79.5
4	415	1490	385	17	118	40	95.3	92.3	90.0
5	572	1480	328	4	118	28	99.1	94.0	91.6

*Average data from last 4 weeks of operation (for reactor No. 4, from last 2 weeks).
†Unfiltered.
‡Filtered.
§Removal efficiency is calculated as the average of removal efficiences for each set of influent and effluent data, and not from average influent and effluent data.

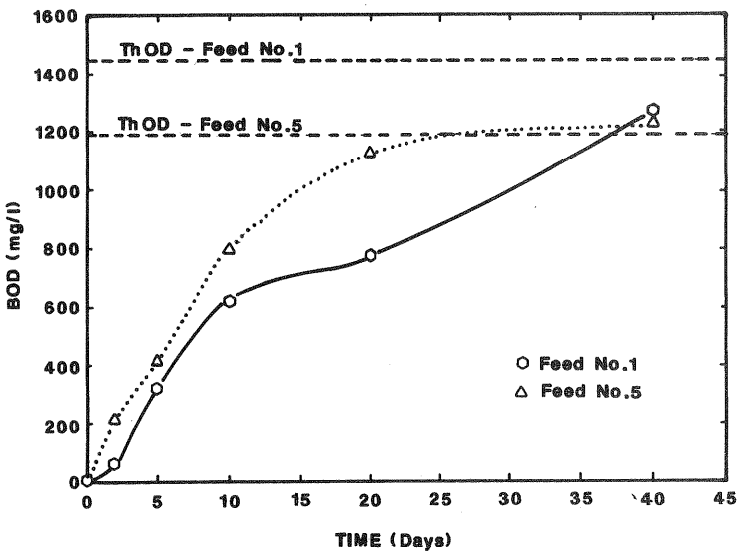

Fig. 2. Results of ultimate BOD tests for selected reactor feeds.

LC_{50} values of the control reactor effluent (No. 5) were, on average, greater than 100%, as were the effluent LC_{50} values from reactor No. 4 which treated a chloride-capped aliphatic surfactant in addition to the cosubstrate. For these types of aliphatic-based products, the acclimated activated sludge was apparently capable of consistently producing non-toxic effluent even at high influent surfactant concentrations of about 600 mg/l and corresponding influent toxicities of less than 1% LC_{50}. Effluents from the three reactors treating nonylphenol-based surfactants were more toxic, with average LC_{50} values from 17 to 58% (Table 5).

Following activated sludge treatment, the average effluent LC_{50} value of reactor No. 2 ($NPE_9OSO_3^-$) was 31%, while that of reactor No. 1 (NPE_9) was 17%. Considering that the cosubstrate in the feed to both units was demonstrated to be untoxic following biological treatment (control reactor No. 5), the differences in toxicities of the tested compounds were reduced from more than an order-of-magnitude difference (0.32% LC_{50} for product A vs 7.7% LC_{50} for product B) to less than a 2-fold difference following biological treatment.

Nonylphenol and low nonylphenol ethoxylates

Nonylphenol and low nonylphenol exthoxylates (NPE_{0-3}) concentrations measured in reactor sludges and effluents are presented in Fig. 3. In general, both effluent and sludge NPE_{0-3} concentrations tended to decrease with time. This pattern can be explained by two different mechanisms—depending on the nature of the respective reactor feed.

In the control reactor No. 5 and in reactor No. 4, the feeds consisted of aliphatic-based products only and should not have contained any NPE_{0-3} or precursors thereof. Small concentrations of constituent(s) with a GC retention time similar to that of NPE_0 (i.e. nonylphenol) were present in the feeds to these reactors. This suspected feed constituent was not identified during the investigations.

NPE_{0-3} in the effluents from reactor Nos 4 and 5 decreased from initial concentrations of 1 mg/l or more to values less than 0.1 mg/l in the last weeks of the study (Fig. 3). Mass balance calculations indicated that the relatively high concentration of NPE_{0-3} found in the effluent during the initial weeks of operation could have been due to gradual elution of these compounds from the seed sludge, which contained 25 mg/l of NPE_{0-3} at the time of seeding.

The feeds to the remaining reactors contained about 60% (organic mass basis) branched, nonylphenol-based products with ethylene oxide molar ratios of 9. All these products are NPE_{0-3} precursors and were present in the feeds at concentrations allowing generation of 155–180 mg/l of NPE_{0-3}, assuming

Table 5. Average performance of the reactors fed with different surfactants*

Reactor No.	CTAS (mg/l)		NPE_{0-3}, (mg/l)		Toxicity, 48 h LC_{50} (%)	
	Influent	Effluent	Sludge	Effluent	Influent†	Effluent‡
1	684	0.91	18.0	0.29	0.29	17
2	518	0.77	10.3	0.25	0.78	31
3	189	0.35	5.6	0.47	0.43	58
4	454	2.45	5.0	0.04	0.14	>100
5	309	0.12	2.1	0.01	0.70	>100

*Average data from last 4 weeks of reactors operation (for reactor No. 4, from last 2 weeks).
†Measured.
‡Harmonic average.

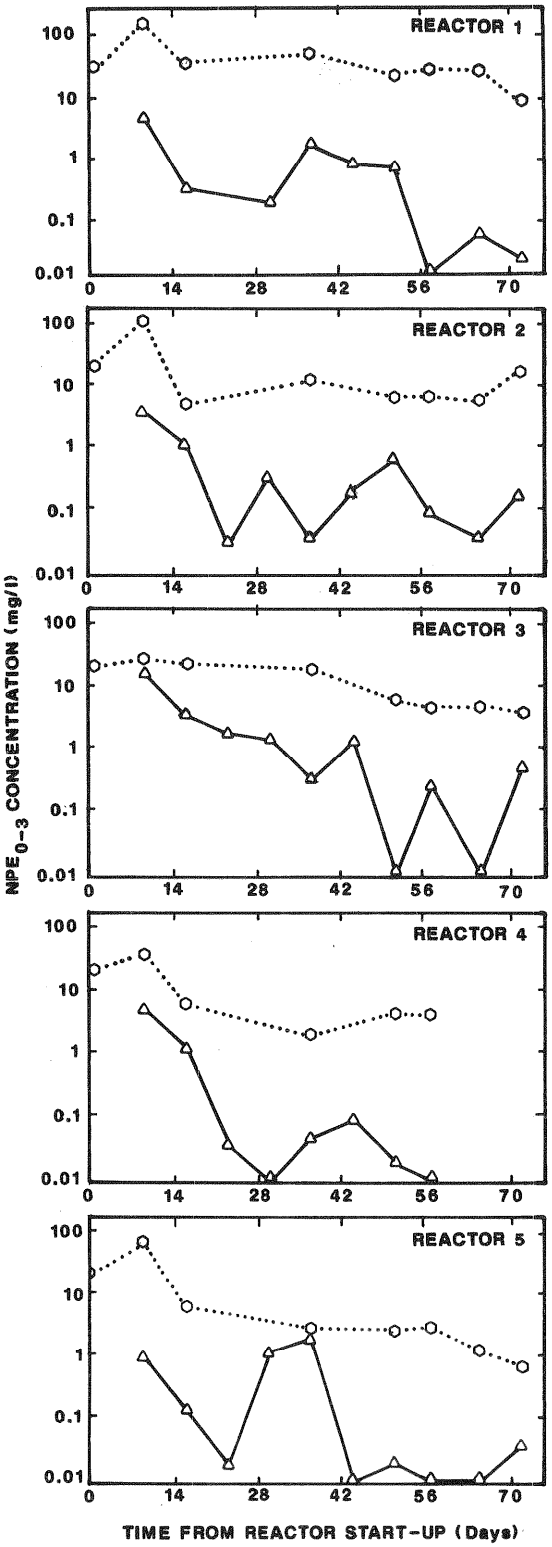

Fig. 3. Low nonylphenol ethoxylates (NPE_{0-3}) concentrations measured in reactor sludges and effluents. ○, Concentration in sludge; △, concentration in effluent.

complete conversion. Effluent NPE_{0-3} concentrations, measured in the last 4 weeks, were below 1 mg/l. The NPE_{0-3} concentrations in the reactor sludges during the last weeks of operation were about 20 mg/l for reactor No. 1, 7 mg/l for reactor No. 2 and 5 mg/l for reactor No. 3.

The concentrations of NPE_{0-3} species on activated sludge samples were apparently attributable to an equilibrium between the rate of primary biodegradation of the products to NPE_{0-3} and related species and the rate of their further (i.e. ultimate) biodegradation. Due to the physical properties of the NPE_{0-3} species, which are highly lipophilic, these and related compounds were expected to have accumulated on the biomass surface, from where they could have been either further degraded or eluted with the effluent. Recently, Brunner *et al.* (1988) found that about 50% (mol/mol) of NPE_n in raw sewage is adsorbed on digested sludge solids in the form of metabolic byproducts (i.e. low ethoxamers).

To determine the extent of NPE adsorption on activated sludge, adsorption isotherm testing was performed for two products, $NPE_{1.5}$ and NPE_9. The $NPE_{1.5}$ product contained a mixture of nonylphenol ethoxamers with an average EO ratio of 1.5 which is equivalent to the species measured by the NPE_{0-3} analytical procedure. A fresh sludge from a municipal treatment plant was first rinsed to remove background organics and was then used in the adsorption experiments. The degree of the NPE_9 product adsorption was determined indirectly by measuring the TOC concentrations of the sludge supernatant after a 10 min contact time at a range of product doses. For the $NPE_{1.5}$ product, adsorption was determined similarly, except the NPE_{0-3} analytical procedure was used to measure $NPE_{1.5}$. The results (Fig. 4) demonstrated that physical adsorption occurred and that the $NPE_{1.5}$ product was more readily adsorbed by the biomass than the NPE_9 product, as had been expected from theoretical considerations. In a separate experiment, the NPE_{0-3} concentration in the supernatant from the adsorption tests was constant over a range of contact times from 1 to 30 min demonstrating that equilibrium was achieved almost instantaneously.

NPE_{0-3} concentrations in the effluents and corresponding sludges from all the continuous-flow

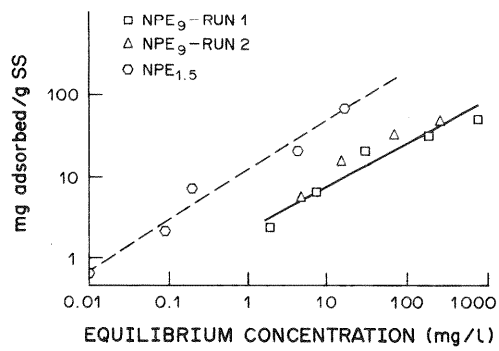

Fig. 4. Adsorption isotherms of nonylphenol ethoxamers on activated sludge (lines indicate linear correlations using logarithms of the values).

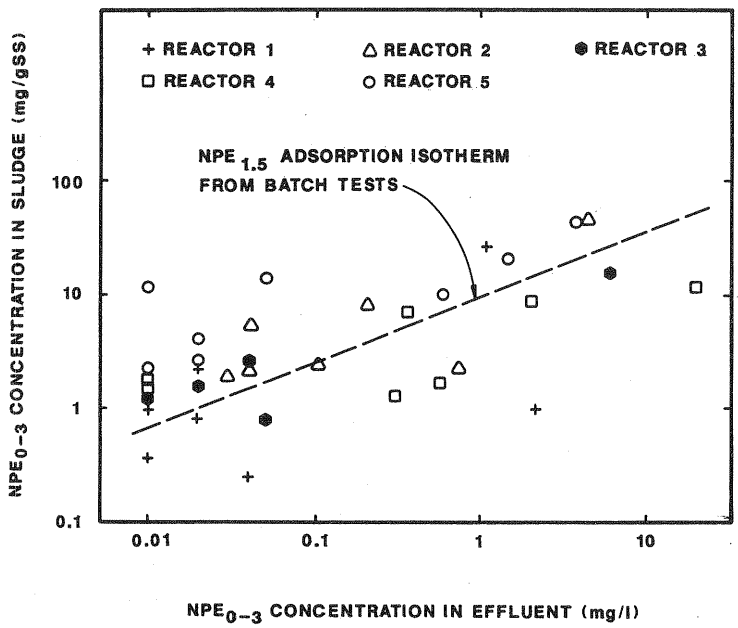

Fig. 5. Correlation of NPE_{0-3} concentrations in sludge and effluent.

reactors are presented in Fig. 5. Despite considerable data scatter, a general equilibrium-type trend is suggested in Fig. 5. The $NPE_{1.5}$ adsorption isotherm from Fig. 4 is also reproduced on Fig. 5 and appears to be applicable to the data, corroborating the importance of adsorption in NPE treatment by the activated sludge process.

Primary biodegradation

While the NPE_{0-3} analytical results provided insights into the formation and fate of biodegradation byproducts, the results of influent and effluent analyses for CTAS were indicative of the extent of primary biodegradation. The CTAS method measured concentrations of ethoxylated compounds with EO chain lengths greater than five.

The average CTAS results from the activated sludge reactors (Table 5) clearly demonstrated that almost complete primary biodegradation occurred in all the reactors. CTAS values were reduced from several hundred mg/l in the influents to less than 1 mg/l for almost all effluent samples tested after the first week of operation.

CONCLUSIONS

Conclusions drawn from the presented results of the activated sludge treatment testing for surfactant biodegradation and mysid toxicity reduction are summarized as follows:

The activated sludge process effectively reduced influent BOD_5 and TOC values for all ethoxylated surfactants tested.

Aliphatic-based products demonstrated slightly better biodegradability in terms of TOC removal efficiency than aromatic-based products.

The results of the CTAS analyses indicated that almost complete primary biodegradation was achieved for all products tested.

Activated sludge treatment substantially reduced influent toxicity for all compounds tested.

Toxicities of the activated sludge reactor effluents were not directly related to the toxicities of the untreated products.

The aliphatic-based products treated in acclimated activated sludge systems produced effluents with low toxicities, even at high influent product concentrations.

Biodegradation of the aromatic-based surfactants produced effluents with markedly higher toxicities than those from the aliphatic-based products.

The presence of an active group at the end of the ethylene oxide chain appeared to have less effect on biological effluent toxicity than did the product base structure (aromatic or aliphatic).

REFERENCES

Ahel M. and Giger W. (1985) Determination of alkylphenols and alkylphenol mono- and di-ethoxylates in environmental samples by HPLC. *Analyt. Chem.* **57,** 1577–1583.

APHA (1985) *Standard Methods for the Examination of Water and Wastewater*, 16th edition. American Public Health Association, Washington, D.C.

Broxterman W. E. and Dean J. C. (1986) Principal players switch roles following mergers, buyouts; technical and market changes mandate reformulations. *Chem. Wk.* May 7, SAS3-43.

Brunner P. H., Capri S., Marcomini A. and Giger W. (1988) Occurrence and behavior of linear alkylbenzenesulphonates, nonylphenol, nonylphenol mono- and nonylphenol diethoxylates in sewage and sewage sludge treatment. *Wat. Res.* **22,** 1465–1472.

Bruschweiler M., Gamperle M. and Schwager F. (1983) Primary biodegradation, ultimate biodegradation, and biodegradation intermediates of alkylphenol ethoxylates. *Tenside* **20,** 317–324.

Conway A. R. and Waggy G. T. (1966) Biodegradation testing of typical surfactants in industrial usage. *Am. Dyestuff Rep.* **55,** 33–40.

Fuka T. and Pitter P. (1980) Relation between molecular structure and biodegradability of organic compounds. IX. Biodegradability of alkylphenolpolyglycol ethers. *Sb. VSChT.* **F23,** 5–45.

Gerike P. and Jasiak W. (1984) Surfactants in the recalcitrant metabolite test. *Surf. Cong. No. 8.* **1,** 195–208.

Hartman L., Wilkerer P. and Staub W. (1967) Reaction-kinetic investigations into the biodegradation of modern surfactants through trickle-filter organisms. *Tenside* **4,** 138–143.

Kravetz L., Chung K. F., Guin K. F., Shebs W. T. and Smith L. S. (1983) Primary and ultimate biodegradation of an alcohol ethoxylate and a nonylphenol ethoxylate under average winter conditions in the U.S. Presented at the American Oil Chemists' Society 74th Annual Meeting, Chicago, Ill.

Lashen E. S., Blankenship F. A., Booman K. A. and Dupre J. (1966) Biodegradation studies of p-t-octylphenoxy-polyethoxyethanol. *J. Am. Oil Chem. Soc.* **43,** 371–376.

Maki A. W., Rubin A. J., Sykes R. M. and Shank R. L. (1979) Reduction of nonionic surfactant toxicity following secondary treatment. *J. Wat. Pollut. Control Fed.* **51,** 2301–2313.

Mann A. H. and Reid V. W. (1971) Biodegradation of synthetic detergents. Evaluation by community trials. Part 2: alcohol and alkylphenol ethoxylates. *J. Am. Oil Chem. Soc.* **48,** 794–797.

Marking L. L. (1985) Toxicity of chemical mixtures. In *Fundamentals of Aquatic Toxicology* (Edited by Rand G. M. and Petrocelli S. R.). Hemisphere, New York.

Osburn Q. H. and Benedict J. H. (1966) Polyethoxylated alkyl phenols: relationship of structure biodegradation mechanism. *J. Am. Oil Chem. Soc.* **43,** 141–146.

Painter H. A. and King E. F. (1978) The effect of phosphate and temperature on growth of activated sludge and on biodegradation of surfactants. *Wat. Res.* **12,** 909–915.

Patterson S. J., Scott C. C. and Tucker K. B. E. (1967) Nonionic detergent degradation. I. Thin-layer chromotography and foaming properties of alcohol polyethoxylates. *J. Am. Oil Chem. Soc.* **44,** 407–412.

Pitter P. and Fuka T. (1979) Biodegradation of non-sulfated and sulfated nonylphenol ethoxylate surfactants. *Envir. Protect. Engng* **5,** 47–56.

Rudling L. and Solyom P. (1974) The investigation of branched NPEs. *Wat. Res.* **8,** 115–119.

Schobert P., Kunkel E. and Espeter K. (1981) Comparative investigations on the microbial metabolism of a nonylphenol and an oxoalcohol ethoxylate. *Tenside* **18,** 64–72.

Stiff M. J., Rootham R. C. and Culley G. E. (1973) The effect of temperature on the removal of non-ionic surfactants during small-scale activated sludge sewage treatment-I. Comparison of alcohol ethoxylates with a branched-chain alkyl phenol ethoxylate. *Wat. Res.* **7,** 1003–1010.

Swisher R. D. (1987) *Surfactant Biodegradation*, IInd edition, pp. 693–719. Dekker, New York.

Sykes R. M. and Rubin A. J. (1979) Treatability of a nonionic surfactant by activated sludge. *J. Wat. Pollut. Control Fed.* **51,** 71–77.

Turner A. M., Abram F. S., Brown V. M. and Painter M. A. (1985) The biodegradability of two primary alcohol ethoxylate nonionic surfactants under practical conditions, and the toxicity of the biodegradation products to rainbow trout. *Wat. Res.* **19,** 45–51.

Yoshimura K. (1986) Biodegradation and fish toxicity of nonionic surfactants. *J. Am. Oil Chem. Soc.* **63,** 1590–1596.

APPENDIX

Application of Joint Toxicity Formulas to LC_{50} values in Wastewater Treatment

The LC_{50} (in %) of a solution in water of an individual compound with a known LC_{50} M (in mg/l) can be calculated from the formula:

$$LC_{50} = (LC_{50}M/C)\cdot 100 \tag{A1}$$

where

LC_{50} = volumetric LC_{50} of the solution (%)
$LC_{50}M$ = compound's LC_{50} (mg/l)
C = compound's concentration in the solution (mg/l).

From the definition of LC_{50}, it can be shown that the toxicity of a mixture of n compounds ($LC_{50_{mix}}$) can be calculated from the following formula (assuming that no synergistic or antagonistic effects are present):

$$LC_{50_{mix}}(\%) = \frac{1}{\frac{1}{LC_{50_1}} + \frac{1}{LC_{50_2}} + \cdots + \frac{1}{LC_{50_n}}} \tag{A2}$$

where

LC_{50_n} = toxicity of a solution of the nth individual constituent at a concentration equal to that present in the mixture (%).

Alternatively:

$$LC_{50_{mix}}(\%) = \frac{100}{\frac{C_1}{LC_{50}M_1} + \frac{C_2}{LC_{50}M_2} + \cdots + \frac{C_n}{LC_{50}M_n}} \tag{A3}$$

where

$LC_{50}M_n$ = toxicity of the nth component (mg/l)
C_n = concentration of the nth component in the mixture (mg/l).

The formulas above can be modified to calculate the LC_{50} of a wastestream resulting from the combination of several wastestreams with known LC_{50}s. The formula has the form of a weighted harmonic mean of the LC_{50}s of the individual streams:

$$LC_{50_{mix}}(\%) = \frac{1}{\frac{f_1}{LC_{50_1}} + \frac{f_2}{LC_{50_2}} + \cdots + \frac{f_n}{LC_{50_n}}} \tag{A4}$$

where

f_n = volumetric fraction of nth stream in the combined stream
LC_{50_n} = LC_{50} of the nth stream (%).

Formulas (A2)–(A4) are valid *only* when no synergistic or antagonistic effects are present.

Finally, to find an average LC_{50} value ($LC_{50_{avg}}$) for a given wastewater stream, the harmonic mean of the available results is calculated according to the formula:

$$LC_{50_{avg}}(\%) = \frac{k}{\frac{1}{LC_{50_1}} + \frac{1}{LC_{50_2}} + \cdots + \frac{1}{LC_{50_n}}} \tag{A5}$$

where

k = number of available results for the average calculation.

Formula (A5) should be used rather than the geometric mean to calculate the average LC_{50} of a wastewater discharge. The average LC_{50} calculated from formula (A5) represents the LC_{50} of a solution which would be obtained by combining equal volumes of the samples being averaged and, therefore, corresponds to averaging the concentrations of pollutants expressed in standard units, i.e. mass per volume. Again, these statements are valid *only* if no synergistic or antagonistic effects were exhibited by such a combination of samples.