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Biological Effects of Surfactants: Part 6—Effects of Anionic, Non-ionic and Amphoteric Surfactants on a Green Alga (*Chlamydomonas*)

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ABSTRACT

The well known algistatic effect of cationic surfactants was not exhibited by homologous series of amphoteric, anionic and non-ionic types assayed with Chlamydomonas reinhardi. *Sulphobetaine amphoterics, 3-(octyl-octadecyldimethylammonio)-1-propanesulphonates, caused growth inhibition when the lipophile contained twelve or more carbon atoms, but only at micellar concentrations. Critical micelle concentration (CMC) of sulphobetaines decreases logarithmically with increasing alkyl chain length and derivatives having eight and ten carbons in the lipophile were below the CMC at the highest concentration tested. Despite their pronounced protein-denaturing properties, sodium higher alkylsulphates did not materially inhibit the growth of* Chlamydomonas. *This may be due to the fact that the Krafft points of these anionics having fourteen or more carbon atoms were above the culture temperatures, thereby reducing their solution properties. Although less inhibiting than the sulphobetains, the non-ionic alcohol ethoxylates decreased growth with increasing hydrophilicity. The most lipophilic non-ionic showed growth-promoting effects.*

INTRODUCTION

Cationic surface active agents, in particular the quaternary ammonium salts derived from higher alkylamines, are well established as microbiocides and algicides. An elaborate series of cationic surfactants, having

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one or more quaternary ammonium radicals, a number of fatty amines and their acetic acid salts, as well as a group of non-quaternary compounds derived from higher alkylamines, have been evaluated for their bacteriostatic, fungistatic and algistatic properties (Hueck *et al.*, 1966). With most compounds 10 ppm, and in numerous cases less than 1 ppm, of the cationic surfactants were required to inhibit the growth of certain Cyanophyta and Chlorophyta.

Little information has, however, been collected on the effects of other classes of surfactant on these organisms. It has been reported that one anionic, sodium laurylsulphate, is toxic to *Spirogyra* at 10 ppm (Andrey & Mirimanoff, 1957). Linear alkyl (C_{12}–C_{13}) benzenesulphonate, also a readily biodegradable anionic, exhibited threshold toxicity to *Selenastrum capricornutum* of 10 ppm whereas only 1 ppm of a non-ionic higher alcohol ethoxylate was required for such an effect (Camp, 1975). Among the green algae, *Chlamydomonas gelatinosa* was found to be more sensitive to alkylarylsulphonates than *Scenedesmus abundans* or *Chlorella saccharophilia* with lethal doses ranging from 70 to 200 ppm (Matulova, 1964).

Toxicity of secondary alkanesulphonates to *Chlamydomonas variabilis* increased in direct proportion with alkane chain length from about ten to about nineteen carbon atoms (Lundahl & Cabridenc, 1978).

A number of investigators have observed that algae in axenic cultures, including Chlorophyta, are capable of degrading surfactants to various degrees, depending on detergent structure and algal species employed (Klein & McGauhey, 1964; Wurtz-Arlet, 1967; Davis & Gloyna, 1969; Camp, 1975; Neufahrt *et al.*, 1978).

The relationship between chemical structure and physical properties of homologous series of anionic, non-ionic and zwitterionic betaine surfactants on algal growth has not been established. Therefore we used the green alga *Chlamydomonas* to screen five homologous series of such detergents.

MATERIALS AND METHODS

Culture methods

Chlamydomonas reinhardi was used in all experiments. Hycel cuvettes (19 × 150 mm) were used as culture vessels. The algae were cultured in

10 ml of Bold's medium (consisting of (in mg litre^{-1}) $NaNO_3$, 250; $CaCl_2 . 2H_2O$, 25; K_2HPO_4, 75; KH_2PO_4, 175; $MgSO_4 . 7H_2O$, 75; NaCl, 25; KEDTA, 81; $FeSO_4 . 7H_2O$, 4·9; H_3BO_3, 11·42) with or without 10 g glucose, to which surfactants were added at concentrations ranging from 0·02 to 2 mM. Cultures were sterilised by autoclaving.

Tubes were inoculated with equal volumes of algal culture. Immediately after inoculation and daily after that for a period of 7–10 days, the absorption of the cultures at 652 nm (OD_{652}) was determined with a Spectronic 70, standardised against the algae-free medium under test. The cultures were maintained under 21 ± 2 °C and 18-h photoperiods provided by a combination of Gro Lux tubes and incandescent bulbs which produced a light intensity of 4·4 mW cm^{-2}. Surfactant-free controls were included with each run. Experiments were replicated six times.

TABLE 1

Composition and Properties of 3-(Alkyldimethylammonio)-1-propanesulphonates (Sulphobetaines)

Lipophile	*Krafft temperature* (°C)	*Molecular weight*	*Tensions in Bold's solution*		
			Conc. (mM)	*Surface* (dynes cm^{-1})	*Interfacial* (dynes cm^{-1})
Octyl (C_8)	0	286	2·0	54·2	19·1
			0·2	68·0	23·0
			0·02	70·5	25·1
Decyl (C_{10})	0	312	2·0	55·0	18·1
			0·2	67·3	21·2
			0·02	71·0	24·2
Dodecyl (C_{12})	0	338	2·0	40·5	5·1
			0·2	51·1	14·9
			0·02	64·0	21·9
Tetradecyl (C_{14})	16	370	2·0	38·7	3·0
			0·2	39·0	4·5
			0·02	43·3	12·2
Hexadecyl (C_{16})	27	399	2·0	39·1	0·5
			0·2	40·0	4·1
			0·02	41·0	7·1
Octadecyl (C_{18})	88	423	2·0	38·8	0·9
			0·2	40·1	6·5
			0·02	44·3	9·0
Control				72·5	25·2

Surfactants

A total of six sulphobetaines having eight to eighteen carbons in the alkyl chain, five sodium alkylsulphates with ten to eighteen carbons in the alkyl group and thirteen non-ionics (Tables 1, 2 and 3) were synthesised and purified as described previously (Bode *et al.*, 1978). The non-ionics consisted of three series of fatty alcohol ethoxylates ranging from ten to eighteen carbons in the alkyl chain and having from about five to about forty ethoxyl units in the hydrophile. These series (Table 3) include surfactants with constant lipophile and varying hydrophiles, constant hydrophile with increasing lipophiles and one wherein the hydrophilic/lipophilic balance (HLB) was held constant, as calculated by the method of Moore & Bell (1956). The comparative water solubility of the ionic sulphobetaines and sodium alkylsulphates can be assessed from the Krafft temperatures (Krafft & Wiglow, 1895) as given in Tables 1 and 2. Surface and interfacial tension (Tables 1, 2 and 3) were measured by the du Nouy method (Schwartz & Perry, 1949; Ernst *et al.*, 1971).

TABLE 2
Composition and Properties of Sodium Alkylsulphates (Anionics)

			Tensions in Bold's solution		
Lipophile	*Krafft temperature* (°C)	*Molecular weight*	*Conc.* (*mM*)	*Surface* (*dynes* cm^{-1})	*Interfacial* (*dynes* cm^{-1})
Decyl (C_{10})	8	260·2	2·0	40·4	12·0
			0·2	53·0	17·9
			0·02	61·1	22·4
Dodecyl (C_{12})	16	288·4	2·0	39·5	4·9
			0·2	53·7	10·0
			0·02	61·4	22·2
Tetradecyl (C_{14})	36	316·4	2·0	37·5	1·1
			0·2	45·0	7·1
			0·02	53·0	14·9
Hexadecyl (C_{16})	44	344·5	2·0	48·3	9·1
			0·2	55·2	15·5
			0·02	57·4	19·0
Octadecyl (C_{18})	56	372·6	2·0	47·6	5·2
			0·2	55·6	12·9
			0·02	59·0	18·8
Control				72·5	25·2

TABLE 3
Composition and Properties of Alcohol Ethoxylates (Non-Ionics)

Lipophile	*Ethoxyl units*	*Hydrophilic/ lipophilic balance*[a]	*Molecular weight*	*Tensions in Bold's solution*		
				Conc. (mM)	*Surface (dynes cm^{-1})*	*Interfacial (dynes cm^{-1})*
Tetradecyl (C_{14})[b]	5·16	36·9	441	2·0	35·5	7·4
				0·2	36·0	9·3
				0·02	38·4	15·8
Tetradecyl[b]	7·11	51·0	527	2·0	31·7	6·4
				0·2	32·3	8·4
				0·02	34·2	15·5
Tetradecyl[b]	10·7	76·4	685	2·0	33·9	6·2
				0·2	34·7	8·9
				0·02	36·3	14·7
Tetradecyl[b]	18·14	129·6	1 012	2·0	41·1	8·7
				0·2	41·0	9·9
				0·02	43·6	15·4
Tetradecyl[b]	40·35	228·2	1 989	2·0	46·2	12·5
				0·2	48·5	14·4
				0·02	49·8	18·8
Decyl (C_{10})	5·07	50·7[c]	381	2·0	36·4	8·5
				0·2	38·8	15·9
				0·02	51·4	21·2
Dodecyl (C_{12})	6·3	52·5[c]	464	2·0	30·1	6·5
				0·2	31·0	11·2
				0·02	37·0	17·3
Tetradecyl (C_{14})	7·11	51·0[c]	527	2·0	31·7	6·4
				0·2	32·3	8·4
				0·02	34·2	15·5

TABLE 3—*contd.*

Lipophile	*Ethoxyl units*	*Hydrophilic/lipophilic balance*[a]	*Molecular weight*	*Tensions in Bold's solution*		
				Conc. (mM)	*Surface (dynes cm^{-1})*	*Interfacial (dynes cm^{-1})*
Hexadecyl (C_{16})	8·18	51·1[c]	602	2·0	36·1	8·5
				0·2	37·9	11·3
				0·02	39·5	14·9
Octadecyl (C_{18})	9·32	51·8[c]	680	2·0	39·4	8·6
				0·2	40·2	15·1
				0·02	44·2	17·2
Decyl (C_{10})	6·70[d]	67·0	453	2·0	29·3	8·5
				0·2	39·9	13·5
				0·02	52·1	16·5
Dodecyl (C_{12})	7·18[d]	59·8	502	2·0	30·4	7·5
				0·2	31·1	10·5
				0·02	39·3	16·1
Tetradecyl (C_{14})	7·11[d]	51·0	527	2·0	31·7	6·4
				0·2	32·3	8·4
				0·02	34·2	15·5
Hexadecyl (C_{16})	7·00[d]	43·8	550	2·0	36·2	8·7
				0·2	37·2	12·1
				0·02	40·1	14·3
Octadecyl (C_{18})	7·00[d]	38·9	578	2·0	39·5	10·0
				0·2	42·9	17·1
				0·02	45·0	18·5
Control					72·5	25·2

[a] $\text{HLB} = \dfrac{\text{Number of ethoxyl units} \times 100}{\text{Number of C atoms in the lipophile}}$. [b] Constant lipophile. [c] Constant HLB. [d] Constant hydrophile.

RESULTS

OD_{652} of control cultures with or without glucose started to increase following the first day, reached a plateau between the sixth and eighth days, and decreased thereafter (Figs. 1 to 5). In the absence of glucose a slightly lower plateau developed in two series (Figs. 1 and 4). In the presence of surfactants, the effect of glucose was not uniform (Figs. 1 to 5). In some instances the OD_{652} was higher and, in others, lower, but in most cases the differences were marginal.

3-(Alkyldimethylammonio)-1-propanesulphonate

When the alkyl group was an *n*-octyl chain, the OD_{652} for all surfactant concentrations was similar to the controls for the first 6 days but decreased after a week (Fig. 1). The OD_{652} was slightly higher on 2 mM concentrations of the surfactant (Fig. 1).

Increases in OD_{652} on the decyl derivative were similar to those on the octyl compounds. However, in the presence of glucose and 2 mM of surfactant, OD_{652} levels were substantially higher between the fourth and sixth days (Fig. 1).

The dodecyl surfactant was inhibitory at 2 mM and somewhat less so at lower concentrations (Fig. 1). All concentrations of the tetradecyl member suppressed increases in the OD_{652} (Fig. 1). In the presence of hexadecyl and octadecyl compounds the suppression of OD_{652} increases was more pronounced than that brought about by the C-12 and C-14 surfactants at all concentrations (Fig. 1).

Sodium alkylsulphates

With the lowest alkyl group in this series, decyl, the OD_{652} pattern during the first 6 days was not appreciably different from the controls. However, the OD_{652} declined more rapidly after the seventh day (Fig. 2).

Patterns with the dodecyl detergent were similar, with a slightly lower OD_{652} value at the highest concentration (Fig. 2).

Except for slightly higher values on glucose-containing media at 0·2 and 0·02 mM, the OD_{652} was moderately lower on the tetradecyl homologue (Fig. 2).

OD_{652} was moderately higher on glucose-containing cultures at all concentrations of the hexadecyl type. The same was true for sugar-free media at 0·02 mM (Fig. 2).

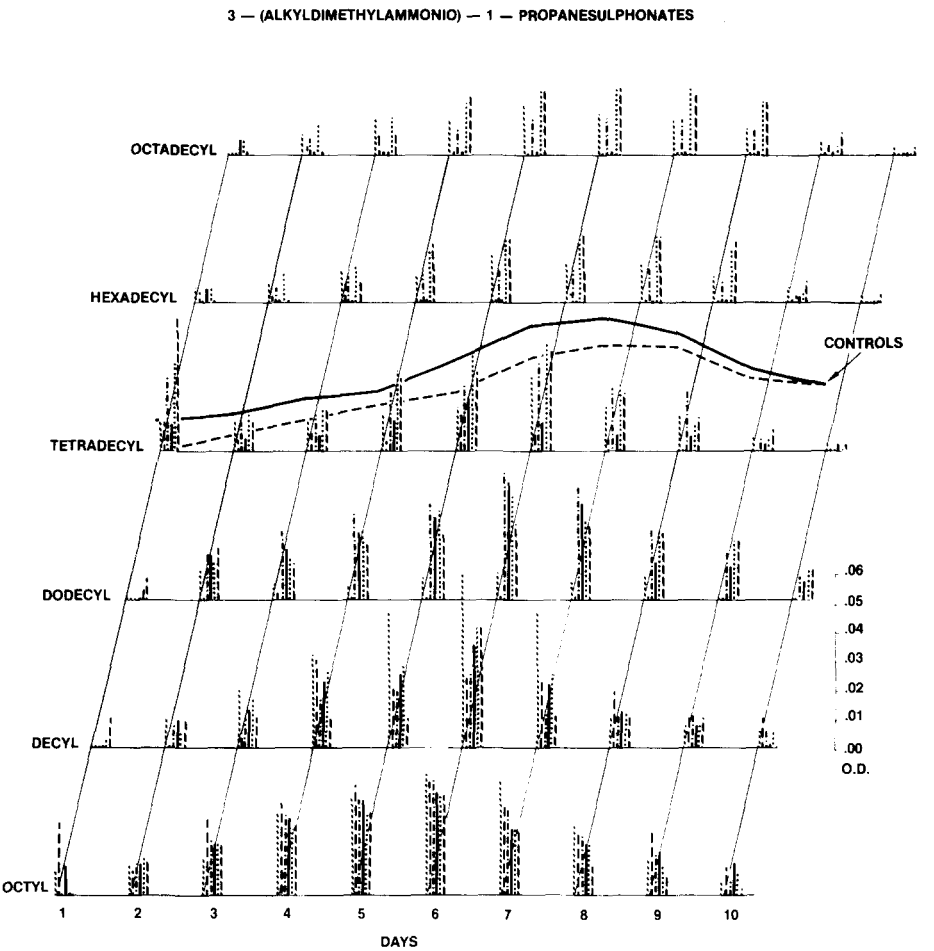

Fig. 1.

Figs 1–5. Optical densities at 652 nm of *Chlamydomonas* grown on glucose-free and 1 % glucose-containing Bold's medium in the presence of 2·0 mM to 0·02 mM concentrations of surfactants. Explanation of symbols: - - - - - - 2·0 mM with glucose; — — — — 2·0 mM glucose free; –·–·– 0·2 mM with glucose; —— 0·2 mM glucose free; · · · · · · 0·02 mM with glucose; – – – – 0·02 mM glucose free. Curves for the controls (solid line for glucose-containing medium and a broken line for sugar-free solution) are superimposed on each Figure.

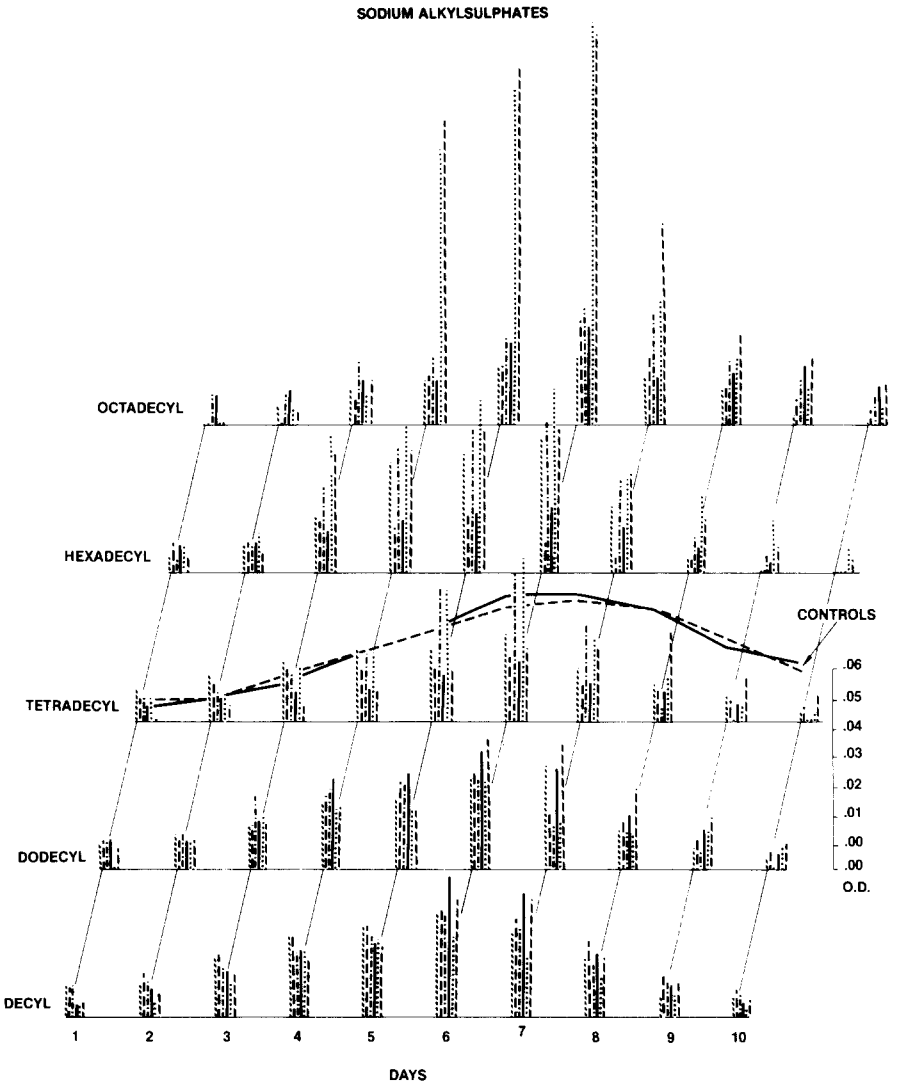
SODIUM ALKYLSULPHATES
OCTADECYL
HEXADECYL
CONTROLS
TETRADECYL
DODECYL
DECYL
.06
.05
.04
.03
.02
.01
.00
.00
O.D.
1
2
3
4
5
6
7
8
9
10
DAYS

Fig. 2.

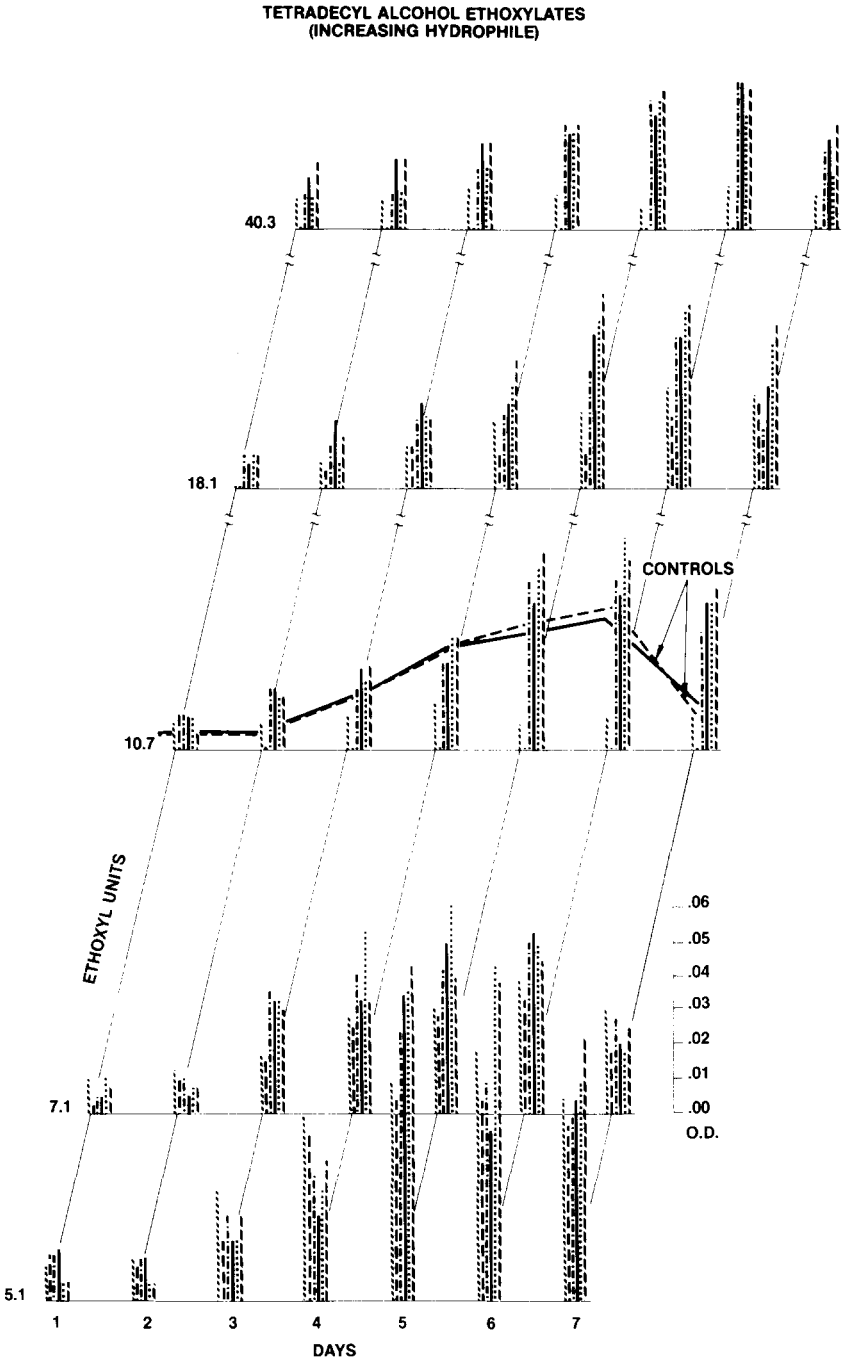
TETRADECYL ALCOHOL ETHOXYLATES
(INCREASING HYDROPHILE)
40.3
18.1
CONTROLS
10.7
ETHOXYL UNITS
7.1
5.1
.06
.05
.04
.03
.02
.01
.00
O.D.
1
2
3
4
5
6
7
DAYS

Fig. 3.

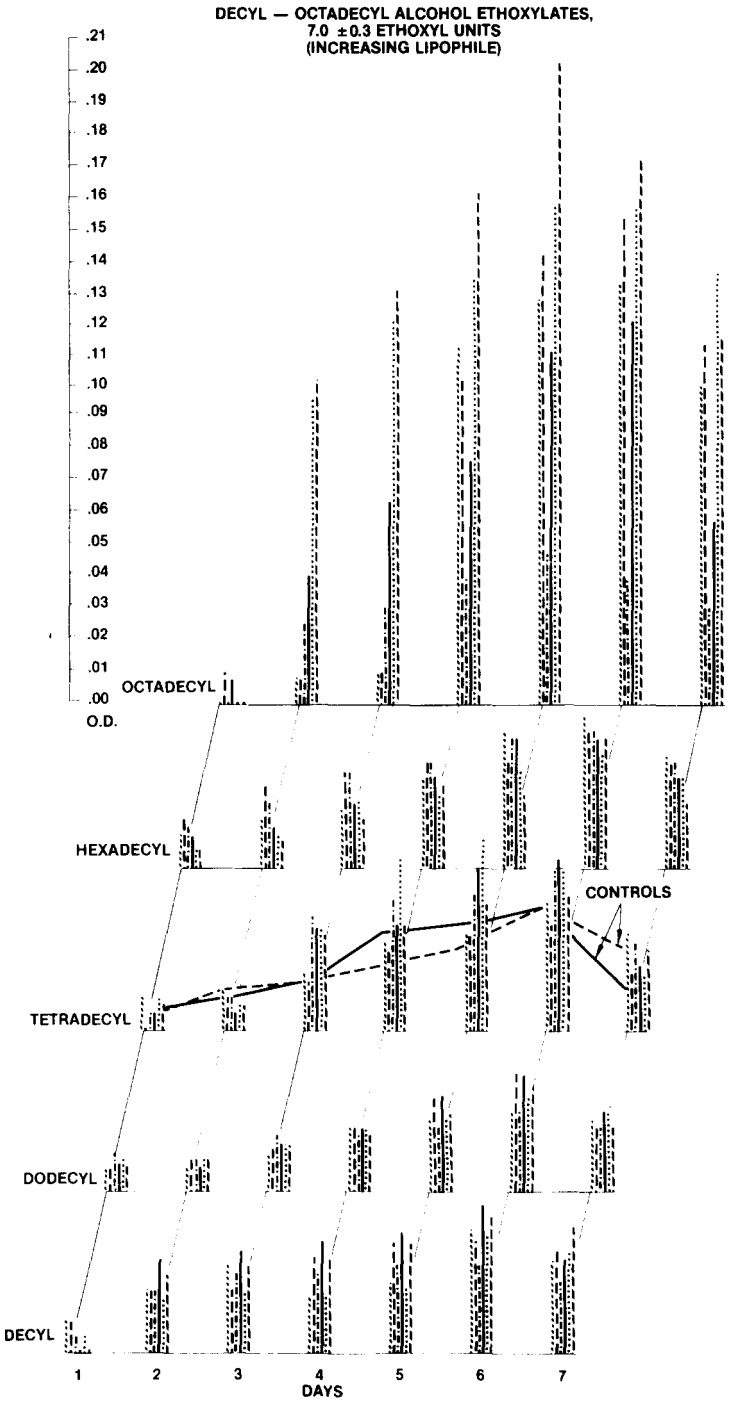
DECYL — OCTADECYL ALCOHOL ETHOXYLATES,
7.0 ±0.3 ETHOXYL UNITS
(INCREASING LIPOPHILE)
.21
.20
.19
.18
.17
.16
.15
.14
.13
.12
.11
.10
.09
.08
.07
.06
.05
.04
.03
.02
.01
.00
O.D.
OCTADECYL
HEXADECYL
CONTROLS
TETRADECYL
DODECYL
DECYL
1
2
3
4
5
6
7
DAYS

Fig. 4.

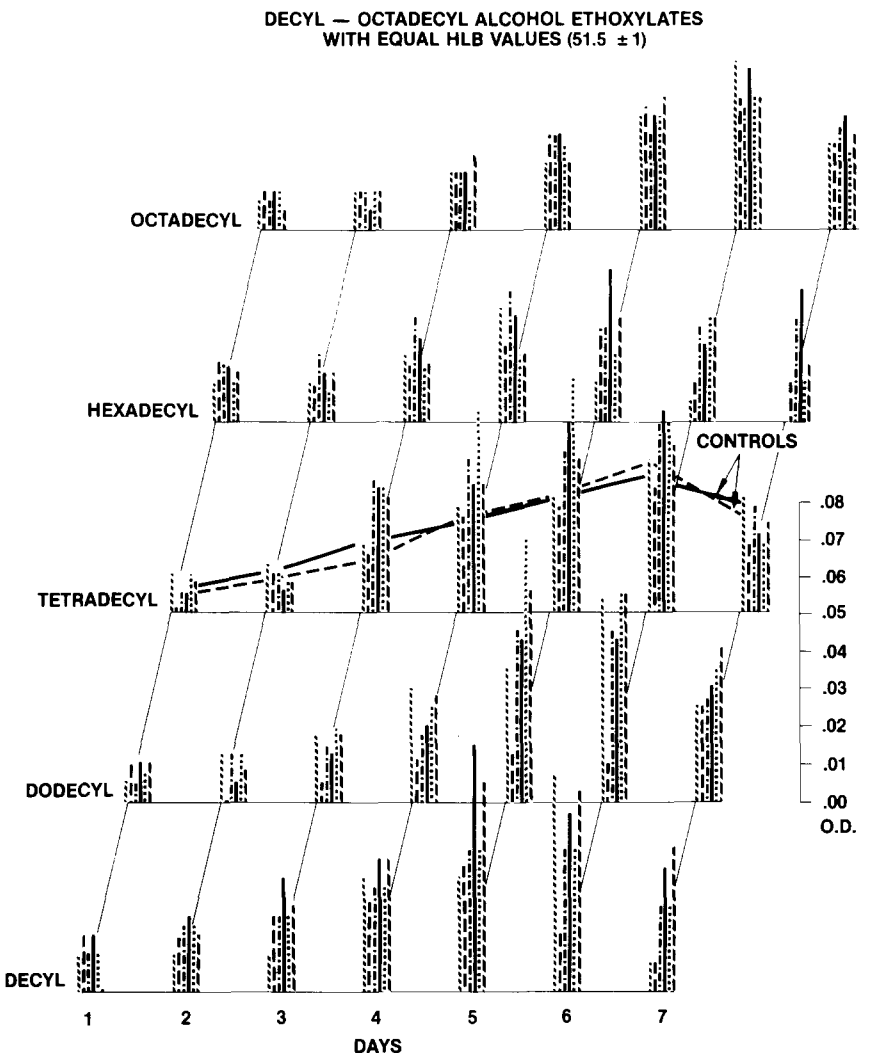

Fig. 5.

The 0·2 mM concentration of the octadecyl derivatives was somewhat inhibitory whereas 0·02 mM brought about notable increases in OD_{652} (Fig. 2).

Tetradecyl alcohol ethoxylates (increasing hydrophile, 5·1–40·3 ethoxyl units)

With all concentrations of the least water-soluble homologue, 5·1 ethoxyl

units, the OD_{652} of the cultures were materially higher than that of the control (Fig. 3).

When the hydrophylic group contained 7·1 ethoxyl units, only 0·02 and 0·2 mM concentrations brought about OD_{652} values greater than controls after the fourth day (Fig. 3).

Cultures on surfactants containing 10·7 ethoxyl units had substantially lower OD_{652} values than controls at the 2 mM level, but the OD_{652} was moderately higher on 0·02 and 0·2 mM (Fig. 3). The more water-soluble members (18·1, 40·3 ethoxyl units) markedly suppressed the OD_{652} of cultures at 2 mM levels. At 0·02 and 0·2 mM the OD_{652} values were similar to the control or marginally higher (Fig. 3).

Decyl–octadecyl alcohol ethoxylates (increasing lipophile, 7·0 ± 0·3 ethoxyl units)

OD_{652} on the least lipophilic (i.e. the most hydrophilic) decyl compound were similar to the control except at the 2 mM level, where they were suppressed (Fig. 4).

A moderate reduction in OD_{652} values occurred on all concentrations of the dodecyl derivative (Fig. 4).

OD_{652} values on 0·02 and 0·2 mM concentrations of the tetradecyl homologue were slightly higher than those of the control. On 2 mM the values were similar to the control or lower (Fig. 4). All OD_{652} values on the hexadecyl surfactant were similar to the controls (Fig. 4).

The least water-soluble surfactant containing an octadecyl group greatly enhanced the OD_{652} at all concentrations and particularly at the lowest one (Fig. 4).

Decyl–octadecyl alcohol ethoxylates (constant HLB values, 51:5 ± 1)

There were no notable overall differences between the OD_{652} on cultures containing this series and the controls (Fig. 5). However, OD_{652} values on the lower concentrations of the decyl and dodecyl derivatives were somewhat higher (Fig. 5). There were no consistent effects of surfactant concentration on OD_{652} (Fig. 5).

DISCUSSION

A correlation between surfactant concentration and toxicity to aquatic organisms has been demonstrated (for a review see Margaritis & Creese, 1979). In our prior work with *Hydra attenuata* (Bode *et al.*, 1978) a

concentration-dependent effect was also noted, employing the same classes of surfactant used in our current study. No effect on the budding rate of *Hydra* was observed below the 0·02 mM level. The range selected for the assay with *Chlamydomonas* (0·02–2·0 mM) consisted of surfactant concentrations within the micellar range as well as below it, where these colloids are present as simple ions or molecules. Thus, at the lowest concentration the ionic compounds were present at 5–8·5 ppm, in line with reported sewage plant effluents (Swisher, 1970), although considerably above the levels observed in river waters where surfactants may be present at barely detectable levels due to dilution and biodegradation. As has been pointed out (Margaritis & Creese, 1979), efficient analytical methods with the required sensitivity, such as the methylene-blue cationic titration of anionic sulphonate or sulphate surfactants, are not available for many other types. This makes assessment of total surfactant levels difficult.

The anionic, non-ionic and amphoteric (sulphobetaine) surfactants assayed with *Chlamydomonas* did not have the powerful algistatic properties which were reported for a broad spectrum of cationics and even some non-betaine amphoterics (Hueck *et al.*, 1966). Higher alkyl sulphobetaines at concentrations in the micellar range retarded growth (as reflected by optical densities). In this homologous series, the critical micelle concentration (CMC) decreases logarithmically with increasing alkyl chain length (Herrmann, 1966). Thus, the octyl and decyl sulphobetaines which had no observable effects on algal growth were not micellar, even at the highest concentration (2·0 mM). The dodecyl derivative was at the threshold or within the CMC only at the 2·0 mM level and only this concentration brought about a markedly lower OD_{652}. With the tetradecyl compound only the lowest concentration was below the CMC. At this concentration the OD_{652} values were not suppressed, in contrast to those of cultures at higher concentrations or to sulphobetaines with longer alkyl chains. The latter were micellar at all concentrations and there was no growth in cultures containing 2·0 mM surfactant in the absence of glucose (Fig. 2).

In common with the potent algicidal cationic quaternary ammonium salts, the sulphobetaines contain a quaternary ammonium ion. This ion is covalently linked to the strongly acidic sulphonate group, giving these compounds ionically balanced properties. Therefore, the ionic interactions exhibited by cationic and anionic surfactants are not shown by this class of zwitterionic compounds. This is also exemplified by the

purification procedure employed (Ernst & Miller, 1982) where impurities are removed from the reaction mass by chromatography through a mixed bed ion exchange resin.

The protein-denaturing properties of sodium alkylsulphate detergents are well documented (Reynolds *et al.*, 1967; Tanford & Reynolds, 1976; Steck & Fox, 1972; Helenius & Simons, 1975; Ernst, 1980). However, these surfactants did not materially inhibit the growth of *Chlamydomonas* at all three concentrations. Sodium decyl- and dodecyl-sulphate were below the CMC even at the highest concentration employed. All higher molecular members had Krafft points which were higher than the culture temperatures (Table 2, Fig. 2). The Krafft point is the temperature at which the solubility of an ionic surfactant becomes equal to the CMC (Krafft & Wiglow, 1895; Rosen, 1978). Solution properties of such surfactants therefore reflect their saturation concentration at the assay temperature. The reduced water solubility of the hexadecyl- and octadecyl-sulphates at these temperatures is no doubt the reason for the negligible effects on cultures which contain them (Fig. 2).

Krafft points are not encountered with non-ionic surfactants which have a negative coefficient of solubility with increased temperature. Water solubility of the tetradecylalcohol ethoxylates (Table 3, Fig. 3) depends on the number of ethoxyl units in their hydrophilic polyethyleneglycol chain. OD_{652} values of these homologues, featuring a constant lipophile and increasing hydrophile, were higher than controls on media containing the two least water-soluble members. The OD_{652} values decreased with increasing water solubility of the surfactants (Fig. 3). This agrees with reports regarding the effects of alkylphenolethoxylates on soybean cell suspension cultures (Davis *et al.*, 1982).

In the series of non-ionics featuring a constant hydrophile, approximately seven ethoxyl units, and a lipophile increasing from twelve to eighteen carbons, the most lipophilic homologue had surprising growth-promoting properties (Table 3, Fig. 4). The high molecular weight lipophile of this member, coupled with its low HLB, led to low water solubility, which resulted in reduced solution properties as substantiated by higher surface and interfacial tensions. However, OD_{652} levels in general could not be correlated with these solution properties. The overall higher growth obtained with more lipophilic members of non-ionic detergents may well be due to a growth promotion such as that reported for fatty esters and alkyl lipids, including non-ionic surfactant esters, on peas (Stowe, 1958, 1960).

Chlamydomonas is capable of heterotrophic growth (Hudock & Fuller, 1966; Levine, 1968). Therefore, a possibility existed that some surfactant effects could be due to the addition of a carbon source rather than their molecular properties. Consequently, all assays were carried out in the presence and absence of glucose to minimise the influence of the added carbon and obtain a better indication of surfactant effects. In addition, the similarity of surfactant effects on media with or without glucose is an indication that the results obtained are not due to the additional carbon.

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