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Toxicity and inhibition of bacterial growth by series of alkylphenol polyethoxylate nonionic surfactants

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ABSTRACT

Laboratory experiments measured the effects of five alkylphenol polyethoxylate nonionic surfactants on the microbial degradation of glucose and pentachlorophenol (PCP) by a pure culture of *Sphingomonas chlorophenolicum* RA2 (RA2) that was unable to biodegrade the surfactants. The surfactants with mid-range hydrophile–lipophile balance (HLB) values of 13.5–15 were the most biocompatible with substrate degradation. Monomers of the surfactant with the lowest HLB value of 12.3 inhibited RA2 growth on both glucose and PCP. The surfactant with the highest HLB of 17.9 was only inhibitory to glucose biodegradation at 3000 mg/L, a concentration well above its CMC. The surfactants were more inhibitory of RA2 biodegradation of PCP compared to glucose, which is likely due to interactions with membrane-associated PCP-degrading enzymes rather than bioavailability limitations. These results may prove helpful in selecting surfactants for use enhancing surfactant-amended remedial applications involving biodegradation or oil dispersion.

1. Introduction

Surfactants are one of the most widely used chemicals, from industry to household use and personal care products. The worldwide estimated consumption of surfactants was 18.2 million tonnes in 2003 [1]. Approximately 2.1 million gallons of dispersants including surfactants were used over two months during the Deepwater Horizon oil spill [2]. Surfactants may be intentionally injected into the subsurface at concentrations above their critical micelle concentration (CMC) in order to enhance the dissolution of organic contaminants as part of Surfactant Enhanced Aquifer Remediation (SEAR). The selection of which surfactant to use for SEAR is generally based on the surfactant which results in the greatest solubilization enhancement of target contaminants, as well as the ability to avoid surfactant losses due to adsorption and biodegradation in the subsurface.

The hydrophile–lipophile balance (HLB) is an important characteristic to consider when selecting a surfactant. The HLB measures a surfactant's affinity between oil and water. For example, HLB 4–5 are water-in-oil emulsifiers, 6–9 are wetting agents, 8–12 are oil dispersants, 10–12 are detergents, and 13–20 are oil-in-water emulsifiers [3]. A high HLB number means that the surfactant is highly soluble in water. Zhou and Rhue [4] found that among 49 surfactants, 12 nonionic surfactants with HLB values between 10.8 and 13.2 resulted in the greatest tetrachloroethene (PCE) solubilization. Nonionic surfactant HLB values that were tested ranged from 7 to 16.9, with decreased efficiency of PCE solubilization noted at lower and higher HLB values.

Because many organic contaminants are biodegradable, potential coupling of bioremediation and SEAR is appropriate to consider [5]. Non-ionic surfactants have been selected as the focus of the current study because they are generally non-toxic to bacteria, have been well studied, and have demonstrated considerable potential for use in remedial applications [6–8]. In addition, alkylphenol polyethoxylate surfactants are often present in municipal and industrial wastewater due to their use in household and industrial detergents. Therefore, it is important to understand how these surfactants impact organic compound degradation by microorganisms in order to promote the use of surfactants that will be compatible with biological treatment of SEAR and municipal wastewaters.

The toxic effects of surfactants on bacteria are largely the result of two mechanisms: (i) direct disruption of the cellular membrane and/or (ii) reactions of the surfactant with enzymes essential to cell functioning. Therefore, although surfactants can increase the bioavailability of low solubility substrates, this benefit may be offset by toxicity and/or micellized substrate being poorly bioavailable. Surfactants with similar chemical structure may exert widely varying effects on bacterial species [9], perhaps due to differences in the HLB. Torres et al. [10] tested the effects of three different nonionic surfactants (SPAN 80, Surfacpol 906, and Tween 80 with HLB values of 4.3, 11, and 15, respectively) on diesel biodegradation by

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Table 1
Properties of tested nonionic surfactants.

Surfactant trade name	Abbreviation	Molecular formula	M.W.	HLB[a]	CMC in DI water (mg/L)	CMC in media (mg/L)	MSR of PCP	Kmw of PCP	Kmw of glucose
Triton X 114	TX114	$C_8H_{17}(C_6H_4)O(CH_2CH_2O)_{7.5}H$	536	12.3	110	NM	0.0032[b]	1.38	
Triton X 100	TX100	$C_8H_{17}(C_6H_4)O(CH_2CH_2O)_{9.5}H$	625	13.5	130[c]; 150	150	0.0041	1.52	0.031
Tergitol NP10	TNP10	$C_9H_{19}(C_6H_4)O(CH_2CH_2O)_{10.5}H$	683	14	50	40	0.0051	1.72	
Igepal CA 720	IG720	$C_8H_{17}(C_6H_4)O(CH_2CH_2O)_{12}H$	735	14.9	160	130	0.0057[b]	1.79	
Triton X 405	TX405	$C_8H_{17}(C_6H_4)O(CH_2CH_2O)_{40}H$	1966	17.9	1600[c]	NM	0.0086	1.01	

[a] HLB calculated as weight %EO/5 adapted from Porter [15]; NM = not measured.
[b] Estimated.
[c] Chao et al. [16].

a mixed consortia of soil bacteria that included five Gram-positive bacterial species and more than three Gram-negative bacterial species; they concluded that the maximum biodegradation rates were observed at a low HLB value of 4.3. Gu et al. [11] found that Triton X 165 (TX165 with an HLB 15.8) was less toxic than Trion X 100 (TX100 with an HLB 13.5) to mixed cultures of microbial cells during biodegradation of acetate and glucose. Yeh et al. [7] explored the effect of HLB on the biodegradation of glucose and lactate by mixed enrichment cultures of anaerobic bacteria, with nine nonionic Tween-surfactants with HLB values of 9.6–16.7 at 200 mg/L. The results showed somewhat increased methane production on lactate with increasing HLB values; however, no strong trend was found. One reason for the unclear results may be due to biodegradation of the Tween surfactant itself (by 14–46%) during the test. Because many nonionic surfactants (such as TX100) can be biodegraded in soil and/or activated sludge by mixed cultures [12,13], this is an important consideration for in situ applications. Alternatively, the chemical structure rather than the HLB may be a more important determinant of the surfactant impact on substrate biodegradation, as was found by Torres et al. [14] during biodegradation of diesel fuel by naturally occurring soil bacteria in microcosm experiments.

In this study, we investigated surfactant effects on the microbial degradation of hydrophilic (glucose) and hydrophobic (pentachlorophenol, PCP) substrates with five different nonionic surfactants (Table 1). These results can be used to improve our understanding of how surfactant properties affect surfactant biocompatibility.

2. Materials and methods

2.1. Biodegradation tests

Sphingomonas chlorophenolicum strain RA2 (RA2) can degrade PCP or glucose as a sole carbon source. RA2 is a gram negative aerobic bacterium that was originally isolated from PCP contaminated soil [17]. RA2 has a moderately hydrophobic cell surface when growing on PCP, based on its measured adhesion to n-hexadecane of $37 \pm 4\%$ during log growth as evaluated using the bacterial adhesion to hydrocarbons (BATH) test method of Rosenberg [18]. By comparison, van Loosdrecht et al. [19] reported the adhesion to n-hexadecane ranged from 0 to ~85% for 18 different bacteria. RA2 was grown on medium adapted from Radehaus and Schmidt [17] containing per liter: 2.4 g of Na_2HPO_4, 2.0 g of KH_2PO_4, 0.1 g of NH_4NO_3, 0.01 g of $MgSO_4{\cdot}7H_2O$, 0.01 g of $CaCl_2$, and 1 mL of trace element solution. The media were sterilized by autoclaving.

The characteristics of the surfactants used in this study are summarized in Table 1. The alkylphenol ethoxylate ether surfactants represent a range of HLB values that are often used in biodegradation studies. RA2 was not able to biodegrade any of the surfactants used in this study, as determined in batch systems with RA2 and the surfactant as a sole carbon source (data not shown and shown previously [20]). All of the surfactants were used as received without purification and diluted with the growth medium solution to selected concentrations.

Batch biodegradation tests were conducted in 250 mL glass Erlenmeyer flasks incubated on a rotary shaker at 160 rpm. The temperature for all experiments was 24 °C (±1 °C). The Erlenmeyer flasks contained 200 mL of sterile growth medium, 100 mg/L of glucose, varying concentrations of surfactant below and above the CMC, and RA2 inoculum. For comparison, biodegradation of a toxic and somewhat hydrophobic substrate, 100 mg/L of PCP (95%, Sigma), was also evaluated. For each test set, inoculated controls with substrate but no surfactant, controls with substrate without RA2 inoculation, and inoculated controls with surfactant but no substrate were evaluated. Each of the experimental conditions was replicated in duplicate or triplicate.

Each bacterial inoculum was obtained from a liquid pure culture of RA2 started from colonies maintained on tryptic soy broth (TSB) plates and then batch grown on 100 mg/L PCP. The flasks were autoclaved and covered with glass wool and aluminum foil to allow for oxygen transfer while preventing contamination. Each flask was inoculated with 5–10% by volume of liquid RA2 culture grown to late log or early stationary phase on PCP. Every 4–12 h, 5–10 mL samples were removed. Biomass was measured by the optical density, absorbance at 600 nm on a Shimadzu UV 160 spectrophotometer. Absorbance was converted to dry mass measured as volatile suspended solid (VSS) by a standard curve [21]. Following centrifugation at 10,000 rpm for 5 min, the supernatant samples were measured for absorbance at 320 nm to indicate the PCP concentration or measured for glucose content by the sulfuric acid–phenol method [22]. These are total concentrations which include both free and micellized substrate.

Kinetic parameters were determined using a spread sheet method (Microsoft Excel solver) which minimized the residual sum of squared errors (RSSE) between the measured and modeled substrate and biomass concentrations over time. Four different models were evaluated: Monod, zero order, first order, and Contois [23]. These methods were previously described in Bielefeldt and Cort [24]. The net yield (Y) was calculated for each test conditions of surfactant concentration based on the measured maximum biomass growth divided by the measured substrate decrease over the same time interval.

2.2. Measurement of surfactant characteristics

The critical micelle concentration (CMC) was determined for these test conditions using a du Nouy ring tensiometer (Fisher Scientific). Samples at the same pH and ionic strength from the biodegradation experiments were mixed at lab temperature (24 ± 1 °C). The CMC was determined by the aqueous surfactant concentration at which the surface tension no longer decreased with increasing surfactant dose.

The effectiveness of a particular surfactant in solubilizing a given solute is known as the molar solubilization ratio (MSR), which can be measured using methods and equations described by Edwards

et al. [25]. The MSR is defined as the number of moles of compound solubilized per the number of moles of surfactant micelles present. In the presence of excess hydrophobic organic compound, the MSR may be obtained from the slope of the curve that results when the solution concentration is plotted against the surfactant concentration. To measure the micelle solubilization of PCP by a particular surfactant, 15 mg of crystalline PCP (1.5 g/L) was added into Teflon caped test vials containing 10 mL of a selected concentration of surfactant-buffered 0.1 M acetic acid (pH = 3.5). Triplicate test vials for each condition were incubated on shaker table rotating at 20 rpm for 24 h. PCP concentrations in centrifuged samples (3600 rpm for 30 min) were measured with a UV spectrophotometer at 320 nm. MSR values for PCP at pH 2 were also previously measured for some surfactants by Cort [20].

3. Results and discussion

3.1. Surfactant characteristics

The characteristics of the five nonionic surfactants used in this study are summarized in Table 1. In this case where the hydrophobic tail moieties are nearly the same, HLB serves largely as an indicator of the effects of the varying degree of ethoxylation in the polar head groups. All of the surfactants used in this study are in a fairly narrow range of HLB values characteristic of oil-in-water emulsifiers that would be likely candidates for use in surfactant-enhanced aquifer remediation at sites containing non-aqueous phase liquids (NAPLs). These commercial mixtures contain a range of different monomer chain lengths, and as such the average values are shown. There was minimal difference in the CMC measured in deionized water (DI) at pH 5.6–6.2 and 24 °C compared to the bacterial growth media at pH 6.8 and 24 °C, as would be expected for nonionic surfactants [26]. The MSR values increase with increasing HLB values. A regression line relating MSR to HLB based on this data (r^2 = 0.988) found that: MSR = 9.75E−4(HLB) − 8.82E−3. This equation was used to estimate the MSR values for Igepal CA 720 (IG720) and Triton X 114 (TX114).

Previous research has shown that the micelle:water partition coefficients (K_{mw}) of compounds can be estimated based on their octanol:water partition coefficient, K_{ow}, and the surfactant properties. The regression line between measured K_{mw} values for 17 organic compounds into TX100 [27] and reported K_{ow} values [28] at 25 °C was:

$$\log K_{mw} = 0.9854 \log K_{ow} + 1.27533$$

Using the measured K_{ow} value for glucose of 1.5×10^{-3} [29] gives an estimated glucose K_{mw} into TX100 micelles of 0.031. As expected, this indicates very minimal partitioning of glucose into surfactant micelles. In addition, the equation is similar to the reported relationship between K_{mw} and K_{ow} reported by Zhang et al. [30] and Valsaraj and Thibodeaux [31]. Similar values are expected for the other non-ionic surfactants tested in this work, due to similar interfacial tension across the micelle:water interface, γ, and micelle radius, r, per:

$$\log K_{mw} = \left[1.00 - 18.0 * \left(\frac{2\gamma * RT}{\gamma}\right)\right] * \log K_{ow} - 2.17 * \left(\frac{2\gamma * RT}{\gamma}\right) + \log\left(\frac{V_o^*}{V_w}\right)$$

where V_o^*/V_w = molar volume of octanol saturated with water/molar volume of pure water = 6.67 [31].

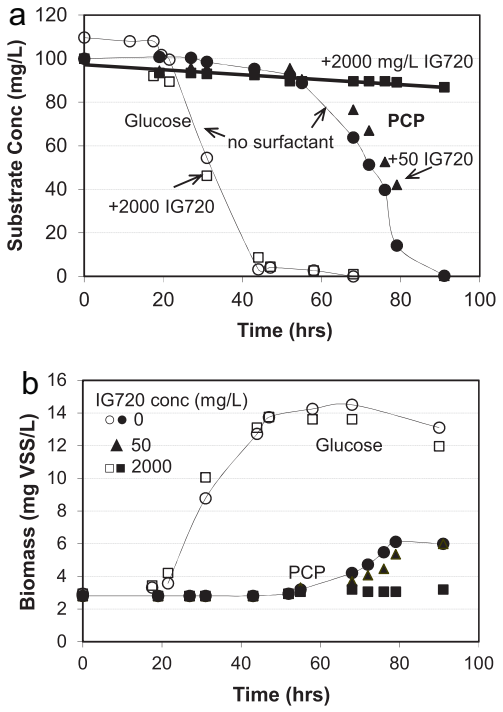

Fig. 1. RA2 growth on glucose(100mg/L) (○,□) and pentachlorophenol (100mg/L) (●,■) with different surfactant concentrations.

3.2. Surfactant impact on glucose biodegradation kinetics

Due to the minimal association with glucose into surfactant micelles, any inhibition of RA2 growth on glucose would be due to surfactant impacts directly on the bacteria rather than interference with substrate bioavailability. Example substrate and biomass concentration versus time data are shown in Fig. 1. These data were used to model the biokinetics; the Monod model was the best fit. Fig. 2 summarizes the effects of different surfactant concentrations on the maximum specific growth rate (μ_{max}) of RA2 on 100 mg/L glucose. The data is presented with the growth rates normalized to the RA2 growth rate without surfactant. The x-axis is shown on a log scale due to the wide range of surfactant doses tested. The error bars represent the standard deviation of the ratio, which was determined from simple error propagation based on the standard deviation of the μ_{max} values.

TX114 caused significant inhibition at low surfactant concentrations, even below its CMC of 110 mg/L. Despite the large error bars, ANOVA of a linear regression of the μ_{max} data versus surfactant

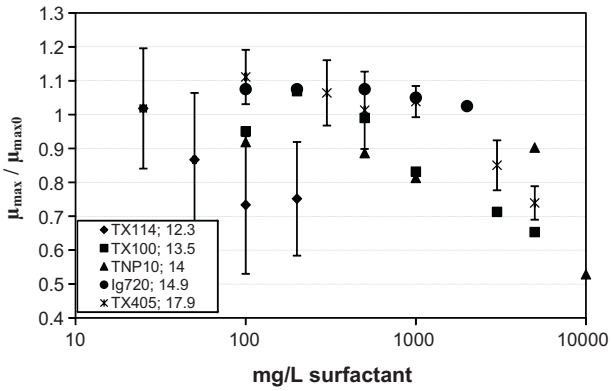

Fig. 2. Normalized maximum substrate growth rate (μ_{max}) of RA2 on glucose.

Table 2
ANOVA linear regression results for surfactant concentration impact on μ_{max}.

Substrate	(HLB)	TX114 (12.3)	TX100 (13.5)	TNP10 (14)	TX405 (17.9)
Glucose	Surfactant concentration range (mg/L)	25–200	200–5000	100–10,000	1000–5000
	Significance *F*	0.0495	0.0303	0.076	7E−5
	R^2	0.401	0.83	0.70	0.91
	Slope $(mg/L)^{-1}\ h^{-1}$	1.18E−4	4.03E−6	3.97E−6	5.89E−6
	Relative std error of slope (RSE)	0.51E−4	1.04E−6	1.49E−6	0.71E−6
PCP	Surfactant concentration range (mg/L)	25–2000	10–1000	20–5000	
	Significance *F*	0.0021	0.0102	0.025	
	R^2	0.616	0.84	0.54	NA
	Slope $(mg/L)^{-1}\ h^{-1}$	1.61E−5	6.19E−5	1.03E−5	
	Relative std error of slope (RSE)	5.20E−6	1.35E−5	3.61E−6	

NA: not available.

concentration showed that the trend with surfactant concentration was significant (significance *F* 0.0495; Table 2). The observed inhibition is presumed to be due to surfactant monomers partitioning into the cell membrane and impairing the barrier function of the membrane and/or interference with cellular enzymes within the cell membrane or inside the cell [32].

IG 720 was the most biocompatible surfactant, causing no inhibition of RA2 growth on glucose at up to 2000 mg/L. TNP10 did not show a trend to increasing inhibition with increasing surfactant concentration over the range of values above the CMC (see Table 2), but at 10,000 mg/L (1%) the μ_{max} was significantly slower. This is presumably due to surfactants forming mixed micelles with membrane lipids resulting in solubilization of the cell membranes.

Low HLB value surfactants are more hydrophobic which tends to interact more with the hydrophobic Gram negative bacterial cell surfaces than more hydrophilic surfactants [33], and therefore causes inhibition of substrate biodegradation by the surfactant. TX405 caused significant inhibition only at concentrations above its CMC. These results are somewhat contradictory to those of Tiehm [34], where toxicity decreased with shorter or longer ethoxylate chains and was related to membrane-damaging effects. He reported that surfactants with ethylene oxide chains composed of fewer than six monomers were buried in the lipid layer of liposomes, and the long ethylene oxide chains (e.g., those with 30 monomers) had no effect on membrane permeability. Gu et al. [11] found that the Triton X 165 surfactant with HLB of 15.8 was less toxic to glucose and acetate degradation than TX100 with an HLB of 13.5. The differences in the cellular responses could be due to the characteristics of the bacteria, such as the cell membranes. However, Gu et al. [11] tested mixed cultures so it is not possible to directly compare the characteristics of the bacteria.

3.3. Surfactant impact on PCP biodegradation kinetics

Fig. 3 illustrates the effect of surfactant concentrations on the μ_{max} of RA2 during biodegradation of 100 mg/L PCP. ANOVA results showed significant inhibition based on increasing surfactant concentrations significantly decreasing the μ_{max} (Table 2). TX114 showed a trend to increasing inhibition at increasing surfactant concentrations, including concentrations below the CMC. The amount of RA2 growth inhibition was similar for similar TX114 concentrations. TX100 and TNP10 were only inhibitory at concentrations above the CMC. IG720 was inhibitory at all tested concentrations, including those below the CMC. IG720 was also the most inhibitory overall.

The n-hexadecane adhesion test [18] was conducted to see if the surfactant TNP10 would change the surface hydrophobicity characteristics of the RA2 cells. During mid log phase growth on PCP without surfactant, 29 ± 5% of the RA2 cells partitioned into the n-hexadecane. RA2 was significantly less hydrophobic when

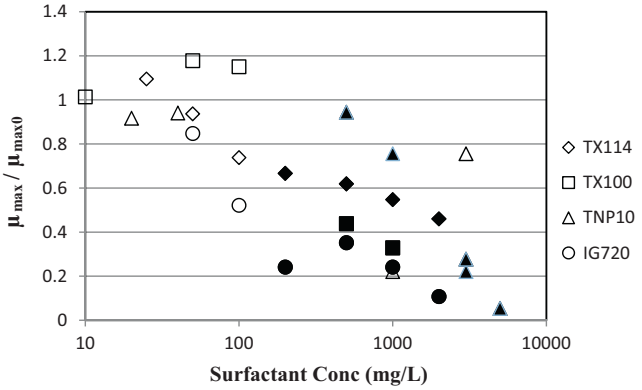

Fig. 3. The effect of surfactant concentrations on the μ_{max} of RA2 during biodegradation of 100 mg/L PCP.

growing on PCP in the presence of 10 mg/L and 100 mg/L TNP10, with only 14 ± 4% and 17 ± 3% adhesion to n-hexadecane, respectively ($p < 0.01$ in 2-tailed, heteroscedastic *t*-test). This indicates some cellular response to the TNP10 surfactant even at 10 mg/L, below where significant changes in the substrate biodegradation were observed. The RA2 cells were also less hydrophobic during the endogenous phase after growth on PCP; 4 ± 3% and 9 ± 7% adhesion to n-hexadecane if 0 or 10 mg/L TNP10 was present, respectively. Changes in cellular hydrophobicity due to bacterial stressors, bacterial substrate, and/or growth phase have also been observed by others [35,36].

One of possible mechanism of surfactant inhibition is membrane fluidity change. Denich et al. [37] reported that pentachlorophenol may change the membrane fluidity of bacteria (UG30), but the effect was not statistically significant. However, combinations of surfactant and PCP may change the membrane fluidity of bacteria. This effect could lower the mass transfer of substrate into the cell.

3.4. Comparison of substrate effects

Fig. 4 compares the maximum specific growth rate ratio of RA2 in the presence of varying surfactant concentrations normalized to the controls without surfactant. Data falling on the 1:1 line indicates that the surfactant caused similar inhibition of RA2 growth on glucose and PCP; this is the case for TX114, and low concentrations of TX100 and TNP10. However, RA2 growth on PCP was significantly more inhibited than its growth on glucose by IG720 and high concentrations of TX100 and TNP10.

The greater surfactant inhibition of PCP degradation compared to glucose degradation could be explained by micellar sequestration reducing bioavailability [38] and/or surfactant interaction with specific substrate-degrading enzymes. While critical PCP

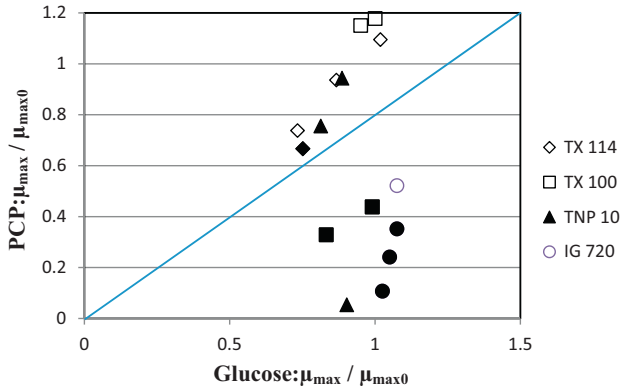

Fig. 4. Comparisons of the PCP versus glucose maximum specific growth rate of RA2 in the presence of varying surfactant concentrations normalized to the controls without surfactant. Open symbols represent surfactant concentrations below the CMC.

degradation enzymes in RA2 are membrane bound [39], glucose degradation enzymes are typically inside the cell wall [40] and therefore likely to be less sensitive to effects from surfactants. Because IG720 and TX114 were inhibitory at concentrations below the CMC, bioavailability cannot account for inhibition and rather enzyme interference by surfactant monomers is suggested. Additional evidence which suggests that lower bioavailability of the micellized substrate was not of primary concern is the fact that a higher TNP10 concentration was required to fully inhibit PCP degradation (~4950 mg/L micelles) than TX100 (~2850 mg/L micelles) despite the fact that more of the PCP is sequestered in the TNP10 micelles than TX100 micelles due to the MSR.

It is important to note that the cell membrane composition of the RA2 cells was significantly different when the cells were grown on glucose versus PCP, based on FTIR analysis using the method of Nichols et al. [41]. In a test using RA2 cells grown without surfactants present, the cells grown on PCP had higher Amide I/CH and Amide II/CH ratios than the cells grown on glucose (data not shown). Other researchers have found cell membrane composition changes with growth substrate [42]. The results indicate that cellular substrate may impact cell membrane composition, and as such the cells may then respond differently to the nonionic surfactants.

3.5. Surfactant effects on cell yield

Cellular toxicity or inhibition will often impact the cell yield. For example, PCP itself is an uncoupler [17] and therefore characterized by low cell yield. The observed cell yield ranged from 0.07 to 0.15 mg VSS/mg glucose compared to 0.03–0.07 mg VSS/mg PCP over the range of experimental conditions. Fig. 5 shows the normalized observed cell yield on glucose and PCP substrates at varying surfactant concentrations normalized to the CMC. At surfactant concentrations below the CMC, all cell yields are at or above the yield on glucose or PCP without surfactant. In fact, significant increases in cell yield on PCP may indicate beneficial effects of the surfactant. Similar effects were previously noted by Cort and Bielefeldt [8]. Above the CMC, TX405 decreases the cell yield on glucose which is an indicator of cellular stress. The only other surfactant that significantly decreased the cell yield was TNP10 on PCP at high concentrations. This is opposite of the effect that occurred at sub-CMC concentrations, indicating different effects from the surfactant micelles. Note that the cell yield in the presence of TX114 could not be calculated because the experiments were conducted above the cloud point of TX114, such that the surfactant interfered with measurement of biomass by optical density.

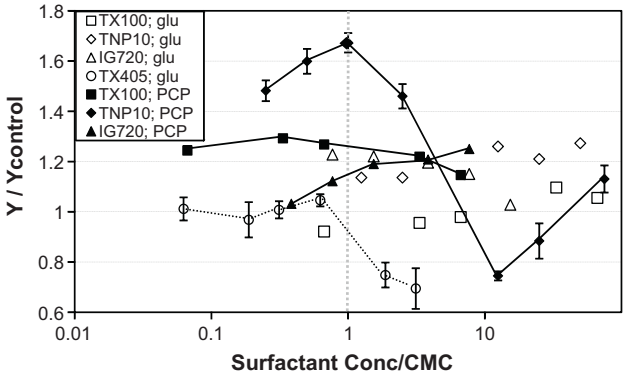

Fig. 5. Normalized observed cell yield on glucose and PCP with surfactants.

In summary, the nonionic surfactants caused mostly favorable changes in observed yield over the range of surfactant concentrations tested, except at high concentrations of TNP10 (PCP) and TX405 (glucose).

4. Summary and conclusions

The effects of nonionic surfactants on the biodegradation of glucose and PCP by a pure culture of aerobic bacteria were investigated. The nonionic surfactants with intermediate HLB values of 13.5–14.9 caused no discernible inhibition of glucose biodegradation at concentrations more than ten times their CMC values. These same surfactants were somewhat inhibitory to PCP biodegradation. This effect is presumed to be due to surfactant interactions with membrane associated enzymes rather than bioavailability limitations. TX114 with the lowest HLB (12.3) of the surfactants tested caused the most inhibition of both glucose and PCP degradation, and these effects occurred at surfactant concentrations below its CMC. The surfactant with the highest HLB value tested (17.9) inhibited glucose degradation only at concentrations above its CMC. The observed cell yields on glucose and PCP were not greatly impacted by the nonionic surfactants.

TX114 appears to be the most toxic because it inhibited both glucose and PCP degradation at low concentrations that were below its CMC. IG720 at a concentration below its CMC inhibited PCP biodegradation, but was not inhibitory to glucose degradation at concentrations up to 15 times the CMC. In fact, the only other nonionic surfactant tested which was found to inhibit glucose biodegradation was Triton X 405. Triton X 100 and Tergitol NP10 were fairly biocompatible with PCP biodegradation, only inhibiting its biodegradation at concentrations above the CMC. The results indicate that lower and higher HLB valued nonionic surfactants are more inhibitory to organic substrate degradation than the nonionic surfactants with mid-range HLB values.

The results obtained in this pure culture study may not be representative of the surfactant impacts on the complex microbial ecology in the subsurface that will be encountered during SEAR. In addition, both substrates tested in this work are fairly hydrophilic, but in SEAR applications with low solubility compounds such as polyaromatic hydrocarbons the impacts of the surfactants on substrate bioavailability may overwhelm the inhibition effects noted in this work. However, nonionic surfactants with mid-range HLB values are good candidates for SEAR application.

References

[1] H.G. Hauthal, CESIO 2004-dynamic surfactants and nanostructured surfaces from innovative industry, SOFW J. 130 (October) (2004) 3–17.
[2] E.B. Kujawinski, M.C. Kido Soule, D.L. Valentine, A.K. Boysen, K. Longnecker, M.C. Redmond, Fate of dispersants associated with the Deepwater Horizon oil spill, Environ. Sci. Technol. 45 (2011) 1298–1306.

[3] L.L. Schramm, Surfactants: Fundamentals and Applications in the Petroleum Industry, Cambridge University Press, Cambridge, UK, 2000.
[4] M. Zhou, R.D. Rhue, Screening commercial surfactants suitable for remediating DNAPL source zones by solubilization, Environ. Sci. Technol. 34 (2000) 1985–1990.
[5] T. Cort, S.K. Park, C. Wozniak, A.R. Bielefeldt, Biotreatment of surfactant flush wastewater from wood treatment soil, J. Environ. Eng. – ASCE 132 (2006) 112–119.
[6] C.A. Ramsburg, K.D. Pennell, L.M. Abriola, G. Daniels, C.D. Drummond, M. Gamache, H.L. Hsu, E.A. Petrovskis, K.M. Rathfelder, J.L. Ryder, T.P. Yavaraski, Pilot-scale demonstration of surfactant-enhanced PCE solubilization at the Bachman road site. 2. System operation and evaluation, Environ. Sci. Technol. 39 (2005) 1791–1801.
[7] D.H. Yeh, K.D. Pennell, S.G. Pavlostathis, Toxicity and biodegradability screening on nonionic surfactants using sediment derived methanogenic consortia, Water Sci. Technol. 38 (1998) 55–62.
[8] T.L. Cort, M. Song, A.R. Bielefeldt, Nonionic surfactant effects on pentachlorophenol biodegradation, Water Res. 36 (5) (2002) 1253–1261.
[9] J.D. Rouse, D.A. Sabatini, J.M. Suflita, J.H. Harwell, Influence of surfactants on microbial degradation of organic compounds, Crit. Rev. Environ. Sci. Technol. 24 (1994) 325–370.
[10] L.G. Torres, N. Rojas, R. Iturbe, Effect of temperature, and surfactant's HLB and dose over the TPH-diesel biodegradation process in aged soils, Process Biochem. 40 (10) (2005) 3296–3302.
[11] J. Gu, G.W. Preckshot, S.K. Banerji, R.K. Bajpai, Effect of some common solubility enhancers on microbial growth, Ann. N. Y. Acad. Sci. 829 (21) (1997) 62–73.
[12] H.J. Chen, S.L. Huang, D.H. Tseng, Aerobic biotransformation of octylphenol polyethoxylate surfactant in soil microcosms, Environ. Technol. 25 (2004) 201–210.
[13] M. Franska, R. Franski, A. Szymanski, Z. Lukaszewski, A central fission pathway in alkylphenol ethoxylate biodegradation, Water Res. 37 (2003) 1005–1014.
[14] L.G. Torres, N. Rojas, R. Iturbe, Use of two-surfactants mixtures to attain specific HLB values for assisted TPH-diesel biodegradation, J. Environ. Sci. 16 (6) (2004) 950–956.
[15] M.R. Porter, Handbook of Surfactants, 2nd ed., Blackie Academic and Professional, Glasgow, UK, 1994.
[16] H.P. Chao, J.F. Lee, C.K. Lee, F.C. Huang, G. Annadurai, Volatilization reduction of monoaromatic compounds in nonionic surfactant solutions, Chem. Eng. J. 142 (2) (2008) 161–167.
[17] P.M. Radehaus, S.K. Schmidt, Characterization of a novel *Pseudomonas* sp. that mineralizes high concentrations of pentachlorophenol, Appl. Environ. Microbiol. 58 (9) (1992) 2879–2885.
[18] M. Rosenberg, Bacterial adherence to hydrocarbons: a useful technique for studying cell surface hydrophobicity, FEMS Microbiol. Lett. 22 (1984) 289–295.
[19] M.C.M. Van Loosdrecht, J. Lyklema, W. Norde, G. Schraa, A.J.B. Zehnder, The role of bacterial cell wall hydrophobicity in adhesion, Appl. Environ. Microbiol. 53 (8) (1987) 1893–1897.
[20] T. Cort, Effects of surfactant on pentachlorophenol biodegradation by *Sphingomonas Chlorophenolicum* sp. Strain RA2. Ph.D. Dissertation. University of Colorado, Boulder, Colorado (2000).
[21] APHA, Standard Methods for the Examination of Water and Wastewater, 21st ed., American Public Health Association (APHA/AWWA/WEF), Washington, DC, 2005.
[22] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28 (3) (1956) 350–356.
[23] B.E. Rittmann, P.L. McCarty, Environmental Biotechnology: Principles and Applications, McGraw-Hill, Boston, 2001.
[24] A.R. Bielefeldt, T. Cort, Dual substrate biodegradation of a nonionic surfactant and pentachlorophenol by *Sphingomonas chlorophenolica* RA2, Biotechnol. Bioeng. 89 (6) (2005) 680–689.
[25] D.A. Edwards, R.G. Luthy, Z. Liu, Solubilization of polycyclic aromatic hydrocarbons in micellar nonionic surfactant solutions, Environ. Sci. Technol. 25 (1) (1991) 127–133.
[26] M.J. Rosen, Surfactants and Interfacial Phenomena, 3rd ed., Wiley-Interscience, Hoboken, New Jersey, 2004.
[27] L. Mokrushina, M. Buggert, I. Smirnova, W. Arlt, R. Schomäcker, COSMO-RS and UNIFAC in prediction of micelle/water partition coefficients, Ind. Eng. Chem. Res. 46 (20) (2007) 6501–6509.
[28] R.P. Schwarzenbach, P.M. Gschwend, D.M. Imboden, Environmental Organic Chemistry, 2nd ed., Wiley-Interscience, Hoboken, New Jersey, 2003.
[29] M.F. Mazzobre, M.V. Roman, A.F. Mourelle, H.R. Corti, Octanol–water partition coefficient of glucose, sucrose, and trehalose, Carbohydr. Res. 340 (6) (2005) 1207–1211.
[30] C. Zhang, G. Zheng, C.M. Nichols, Micellar partitioning and its effects on Henry's law constants of chlorinated solvents in anionic and nonionic surfactant solutions, Environ. Sci. Technol. 40 (1) (2006) 208–214.
[31] K.T. Valsaraj, L.J. Thibodeaux, Relationships between micelle–water and octanol–water partition constants for hydrophobic organics of environmental interest, Water Res. 23 (2) (1989) 183–189.
[32] J.L. Li, B.H. Chen, Effect of nonionic surfactants on biodegradation of phenanthrene by a marine bacteria of *Neptunomonas naphthovorans*, J. Hazard. Mater. 162 (2009) 66–73.
[33] C. Tanford, The Hydrophobic Effect: Formation of Micelles and Biological Membranes, 2nd ed., John Wiley and Sons, New York, New York, 1980.
[34] A. Tiehm, Degradation of polycyclic aromatic hydrocarbons in the presence of synthetic surfactants, Appl. Environ. Microbiol. 60 (1) (1994) 258–263.
[35] N. Christova, B. Tuleva, Z. Lalchev, A. Jordanova, B. Jordanov, Rhamnolipid biosurfactants produced by *Renibacterium salmoninarum* 27BN during growth on n-hexadecane, J. Biosci. 59 (2004) 70–74.
[36] G. Mohanty, Surface Hydrophobicity of Naturally Occurring Bacterial Cultures Degrading Aliphatic Hydrocarbons and Diesel Oil: Characterization and Impacts on Growth and Degradation, Ph.D. Dissertation, Indian Institute of Technology, Bombay, 2006.
[37] T.J. Denich, L.A. Beaudette, M.B. Cassidy, H. Lee, J.T. Trevors, Membrane fluidity of the pentachlorophenol-mineralizing *Sphingomonas* sp. UG30, J. Fluoresc. 13 (5) (2003) 385–391.
[38] F. Volkering, A.M. Breure, W.H. Rulkens, Microbiological aspects of surfactant use for biological soil remediation, Biodegradation 8 (6) (1998) 401–417.
[39] S.D. Copley, Evolution of a metabolic pathway for degradation of a toxic xenobiotic: the patchwork approach, Trends Biochem. Sci. 25 (6) (2000) 261–265.
[40] D.L. Nelson, M.M. Cox, Lehninger Principles of Biochemistry, 4th ed., W. H. Freeman Publisher, 2004.
[41] P.D. Nichols, J.M. Henson, J.B. Guckert, D.E. Nivens, D.C. White, Fourier transform-infrared spectroscopic methods for microbial ecology: analysis of bacteria, bacteria–polymer mixtures and biofilms, J. Microbiol. Meth. 4 (1985) 79–94.
[42] T.J. Denich, L.A. Beaudette, H. Lee, J.T. Trevors, Effect of selected environmental and physico-chemical factors on bacterial cytoplasmic membranes, J. Microbiol. Meth. 52 (2003) 149–182.