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Nutrient and surfactant enhancement for the biodegradation of chlorinated hydrocarbons in the wastewater from a Louisiana Superfund site

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Abstract

Surfactant based soil washing and flushing is an emerging technology for Superfund site remediation in the United States. The presence of surfactants in the wastewater, however, poses challenging problems for subsequent biological or physical–chemical processes. The objective of this research is to evaluate the potential effects of selected surfactants on the biodegradation of chlorinated hydrocarbons in the wastewater from the Petro Processors (PPI) Superfund site north of Baton Rouge, LA. Results from this study showed that biodegradation of a real world waste containing a broad array of hazardous contaminants was significantly enhanced by the amendment of mineral nutrients and surfactants, especially a nonionic surfactant Witconol. The enhancement based on TOC reduction was 49% higher for the mixture of PPI wastewater with Witconol than the combined biodegradation of PPI wastewater and Witconol alone, whereas a similar enhancement was observed with an anionic surfactant sodium dodecylsulfate (SDS). The addition of mineral nutrients was also shown to further enhance the biodegradation of PPI wastewater, with a 13% increase in TOC reduction as compared to the nutrient limited controls. Nutrient addition significantly increased microbial growth, biodegradation, and foam degradation of surfactant-laden PPI wastewater. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

The clean up of contaminated soil and groundwater is slow and costly by current pump-and-treat (PAT) remediation. Surfactant-based technology holds the potential to improve conventional PAT by increasing both the solubilization and mobilization of contaminants with normally very low aqueous solubilities and high interfacial tensions. The U.S. EPA recognizes this as one of the promising innovative technologies applicable to Superfund sites [1]. Considerable research has been done in the past years on soil washing and flushing using surfactants [2–8]. Both laboratory and field experiments have shown the enhanced efficiency for the clean up of contaminated soils. However, little information is available on the aboveground treatment of surfactant-laden wastewater generated from soil washing processes at a real world hazardous waste site. The presence of high concentrations of surfactants may result in foaming, cause operational problems, and render air stripping unsuccessful for the removal of volatile contaminants. High concentrations of surfactants may also reduce the efficiency of activated carbon processes, or possibly limit biological processes due to the toxicity of some synthetic surfactants and their intermediate metabolites.

Few studies to date have addressed the treatment of surfactant-laden wastewater using non-biological means. Ellis and Payne [2] noted the difficulties in treating aqueous surfactant-contaminant effluent, and indicated that separating surfactants from the contaminants for recycle is a vital step for future cost-effective applications in the development of surfactant flushing remediation. Physical–chemical methods such as hydrolysis, oxidation and precipitation require severe conditions [9]. Foam fractionation and adsorption are not feasible due to high concentrations of surfactants [10]. Gannon et al. [11] treated surfactants loaded with *p*-dichlorobenzene (DCB) using gentle extraction with hexane, and the recovered surfactant was recycled for further soil washing. The extraction procedure, however, can only recycle ionic surfactants. In addition, the cost-effectiveness of this technology for a large-scale application is questionable. More recently, Maillacheruvu et al. [12] found that UV-photocatalysis reduced the foaming potential of various surfactants in addition to breaking down an aromatic compound, viz., naphthalene.

Bioremediation is an attractive alternative for the treatment of such wastes. However, the presence of surfactants will complicate the biological process due to the interactions between surfactant, hydrophobic organic compounds (HOCs) and microorganisms. There are conflicting results on the effects of surfactants on biodegradation of HOCs. A number of researchers indicated surfactant enhancement of the microbial degradation of organic contaminants [13,14]. Such enhancement could be due to increased solubility and hence the increased bioavailability of HOCs to the microorganisms, the increased hydrophobicity of the cell membrane [15], or reduced interfacial tensions, thus promoting more contact area between HOCs and microorganisms. The enhanced biodegradation by surfactants is of significance since most HOCs of environmental interest are characterized by long half-lives [16]. On the contrary, other studies indicated the ineffectiveness or even inhibitory effects of surfactants on the biodegradation of HOCs [17,18].

The overall objective of this study is, therefore to investigate the effectiveness of bioremediation for the treatment of organic contaminants in a surfactant-laden wastewater collected from a local Superfund site. In this paper, we address the potential enhancement for the biodegradation of HOCs present in the wastewater. The specific objectives are: (1) to determine the biodegradability of contaminants in the PPI wastewater by acclimated microorganisms, (2) to determine the effects of nutrient amendment on the biodegradation of a real world wastewater from a Superfund site, and the biodegradation of the same wastewater in the presence of surfactants, and (3) to investigate the potential enhancement of biodegradation of petroleum hydrocarbons in the presence of surfactants.

2. Experimental procedure

2.1. PPI Superfund site description

The Petro Processors (PPI) Superfund site is one of the 1228 sites on the EPA National Priority List (NPL) [19]. The site, with an area of 25 hectares, is located 16 km north of Baton Rouge, LA. During the period from 1964 to 1979, a number of industries within the area disposed their toxic sludge, industrial waste and debris in unengineered and uncontrolled pits. In total, approximately 320 000 tons of waste were disposed of at the site. The total volume of deposits contaminated with this waste was estimated to be 1 million m^3. A detailed site description can be found elsewhere [20].

Current remediation projects on the PPI site include a hydraulic containment/recovery system, incineration of organics in the liquid and vapor streams, and air stripping and activated carbon for effluent treatment. The major problem related to the hydraulic containment and recovery system is the inefficiency for the removal of non-aqueous phase liquids (NAPLs) over time. Excessively long periods of clean-up time are predicted for the current scenario [21]. Therefore, a large-scale in situ and ex situ demonstration project using micro-gas surfactant dispersions called colloidal gas aphrons (CGAs) is being investigated [22,23]. As a part of the overall project, the work presented here investigates the effectiveness of biodegradation for the treatment of surfactant-laden wastewater as an alternative between contaminant recovery and air stripping/activated carbon processes which are currently in use at the site, in order to ascertain the viability of CGA use in the current remedy.

2.2. Characteristics of the wastewater

A composite wastewater sample was collected from the PPI site and was used for this study. The wastewater has a brown color with a petrochemical odor. Contaminants include hexachlorobutadiene (HCBD), hexachlorobenzene (HCB), trichloroethylene (TCE), halogenated organic solvents (1,2-dichloroethane (DCE), tetrachloroethane), volatile aromatic hydrocarbons including benzene and toluene, and polynuclear aromatic hydrocarbons [24]. A typical wastewater sample was analyzed by GC/MS, and the concentrations and properties [16] of primary organic contaminants are given in Table 1.

Table 1
Concentrations and properties of selected organics in the PPI wastewater

Chemical	Conc. ($\mu g l^{-1}$)	Solubility ($mg l^{-1}$)	Aerobic Half-Life[a]
Benzene	< 2500	1780	5–16 days
Carbon tetrachloride	4260	800	6 months–1 yr
bis(2-Chloroisopropyl) ether	2640	1700	18 days–6 months
1,1-Dichloroethanes	5720	5500	32 days–22 weeks
1,2-Dichloroethanes	380 000	8690	100 days–6 months
Hexachlorobenzene	< 100	0.005	2.7–5.7 yr
Hexachlorobutadience	207	3.2	4 weeks–6 months
Hexachloroethane	116	50	4 weeks–6 months
Naphthalene	323	880	20 days
Tetrachloroethylene	6930	150	6 months–1 yr
Trichloethanes	790 000	4500	4.5 months–2 yr
Trichloethene	14 400	1100	6 months–1 yr
Vinyl chloride	< 2500	1100	4 weeks–6 months

[a]Unacclimated aqueous biodegradation [16].

2.3. Seed acclimation, culture medium and aerobic reactor

An activated sludge sample was obtained from the Central Municipal Wastewater Treatment Plant (CMWTP) in Baton Rouge, LA. The sample was aerated for 24 h and part was withdrawn from the aerated sludge for transfer to the basal salt media (BSM). Wastewater from the PPI site and a mixture of test surfactants were added in different amount to a series of 250-ml autoclaved Erlenmeyer flasks. The flasks were shaken on a reciprocal shaker at room temperature ($25 \pm 2°C$). The culture was transferred weekly by taking 1 ml of inoculum into a new series of flasks containing BSM, and higher concentrations of test surfactants and PPI wastewater were added. The microorganisms were thus acclimated to surfactants and toxics found at the PPI site. Using the acclimated microorganisms, aerobic shaker-flask experiments were conducted in duplicates in 250-ml autoclaved Erlenmeyer flasks containing test surfactants, the wastewater from the site, or the mixture of surfactants and wastewater. The basal salt medium (BSM) for the aerobic study was prepared by dissolving 5.8 g K_2HPO_4, 4.5 g KH_2PO_4, 2.0 g $(NH_4)_2SO_4$, then adding 0.05 M $MgCl_2$ (0.19 $mg l^{-1}$), 0.1 M $CaCl_2$ (0.022 $mg l^{-1}$), 0.25 M $MnCl_2$ (0.252 $mg l^{-1}$) and 0.01 M Na_2MoO_4 (0.041 $mg l^{-1}$) in 1 l of deionized water (modified from [25]). The medium was filter-sterilized before use.

2.4. Aerobic biodegradation experimental design

For the biodegradation of PPI wastewater containing chlorinated and non-chlorinated hydrocarbons, 4 types of treatments were employed to examine the effects of nutrients and inoculation of acclimated seeds: (a). PPI wastewater + BSM + seed; (b). PPI wastewater-BSM + seed; (c). PPI wastewater + BSM-seed; (d). PPI wastewater + BSM + seed + NaN_3. Sodium azide (NaN_3) was used to kill the microorganisms, hence

treatment (d) is an abiotic control to account for possible loss due to vaporization, evaporation, and adsorption. This non-biological loss was shown to be insignificant.

For the biodegradation of surfactant-laden PPI wastewater, either one of the individual surfactants (anionic sodium dodecylsulfate SDS, or nonionic alcohol ethoxylate Witconol SN-90) was added to the PPI wastewater. The mixture was amended with and without the nutrients to examine the nutrient effects on biodegradation. The treatments were: (e). Surfactant + PPI wastewater + BSM + seed; (f). Surfactant + PPI wastewater-BSM + seed; In addition, another treatment with pure surfactant at the same concentration was included, that is, (g). Surfactant + BSM + seed. The biodegradation of the 'mixture' (e) was then compared with the 'combined' biodegradation (a + g) while the surfactant (g) or the wastewater (a) separately serves as the only carbon source. This experiment was designed to determine the possible interactions between surfactants, organic contaminants and microorganisms. It should be noted that the BSM medium provided mineral nutrients only, and the carbon from the addition of seeds is also negligible.

The percent biodegradation for the 'mixture' was calculated as follows:

$$\%\text{Biodegradation} = \frac{(TOC_{0,e} - TOC_{t,e})}{TOC_{0,e}} \times 100 \tag{1}$$

The percent biodegradation for the 'combined' was calculated using Eqs. (2) and (3):

$$\%\text{Biodegradation} = \frac{\Delta TOC}{TOC_{0,a} + TOC_{0,g}} \times 100 \tag{2}$$

where the change of TOC over time is:

$$\Delta TOC = \left(TOC_{0,a} + TOC_{0,g}\right) - \left(TOC_{t,a} + TOC_{t,g}\right) \tag{3}$$

In Eqs. (1)–(3), the first subscript $(0, t)$ denotes the time, and the second subscript (a, e, g) denotes the treatment as described above.

2.5. Measurements of biodegradation parameters

Biodegradation was monitored over time by measuring microbial growth, surfactant concentrations, foaming potential of residual surfactants, and total organic carbon (TOC). Microbial growth was measured by either optical density at 540 nm or protein contents (Bio-Rad Detergent Compatible Protein Assay, modified from [26]). If optical density was used, the data were corrected from the background color absorbance and then correlated with cell mass. Primary biodegradation was measured by the methylene blue active substance (MBAS) method for anionic surfactants, or the cobalt thiocyanate active substance (CTAS) method for nonionic surfactants [27]. Foam potential was measured using a column apparatus modified from Nelson et al. [28]. Samples were diluted to a final volume of 50 ml, and placed in a 250-ml graduated cylinder. The diluted surfactant solution samples were bubbled with air at a flowrate of 100 ml min^{-1}. Air was introduced at the bottom through a sintered glass diffuser of fine porosity. Net foam volumes (difference between the upper foam level and the foam solution interface)

were recorded at 60 s. TOC was measured as total organic carbon (TOC) or dissolved organic carbon (DOC) or both, by a Model TOC-500 Analyzer with an ASJ-502 auto sampler injection (Shimadzu, Kyoto, Japan). TOC, measured without centrifuging the samples, refers to the sum of DOC and particulate (cellular) organic matter (POC). DOCs were measured after samples were centrifuged. The difference (TOC–DOC) is directly related to the carbon in the biomass, which is an estimate for the biomass contribution to the total TOC removal. For SDS, however, precipitation occurred in the medium. Addition of EDTA was reported to overcome the precipitation problem in another biodegradation study [9], but it was not effective possibly due to the high concentration of surfactant used in this study. Hence, only TOC values are reported for SDS so that the percent biodegradation data will not be affected by the formation of precipitates. TOC and DOC are used as gross parameters to represent biodegradation of either surfactants or organic contaminants in the wastewater. It is a more conservative estimate of biodegradation than individual compounds since TOC values decrease only when compounds are completely transformed into CO_2. In addition, previous studies indicated that standard analyses based on GC and GC/MS often miss the more polar and nonvolatile organic compounds, and quantify a small percentage (less than 1%) of the TOC [29–31]. Therefore no effort was made to distinguish the biodegradation of surfactants from that of contaminants in the mixture, or to determine degradation of specific compounds due to the very complex nature of wastewater from the Superfund site.

2.6. Statistical analysis

Multiple comparisons between the means of each treatment at a given sampling time were performed with Fisher's Least Significant Difference (LSD) test. Wilcoxon Rank Sum test was used for the comparisons of means in two independent groups (treatments). This nonparametric method was selected because the normality assumption was not satisfied for the time series data. All data were analyzed with SPSS statistical software (SPSS). Statistical significance was considered at a level of 5% ($p < 0.05$).

3. Results and discussion

3.1. Biodegradation potential of PPI wastewater

3.1.1. Effects of nutrient addition and acclimated seed

Fig. 1 shows the effects of mineral nutrients (BSM) and acclimated seed on the biodegradation of PPI wastewater, the killed control was not shown since no significant biodegradation was observed ($< \pm 5\%$ DOC reduction). The initial DOC (TOC) value of the wastewater was 750.4 ± 13.7 mg l^{-1} (829.0 ± 2.8 mg l^{-1}), and the % biodegradation was calculated based on this DOC value. The biodegradation under both nutrient amendments and inoculation (treatment a: BSM, seed) was between $21.5 \pm 1.8\%$ (3 days) and $33.9 \pm 5.6\%$ (20 days), while the biodegradation without nutrient amendments but inoculation (treatment b: no BSM, seed) was between $11.1 \pm 2.2\%$ (3 days) and
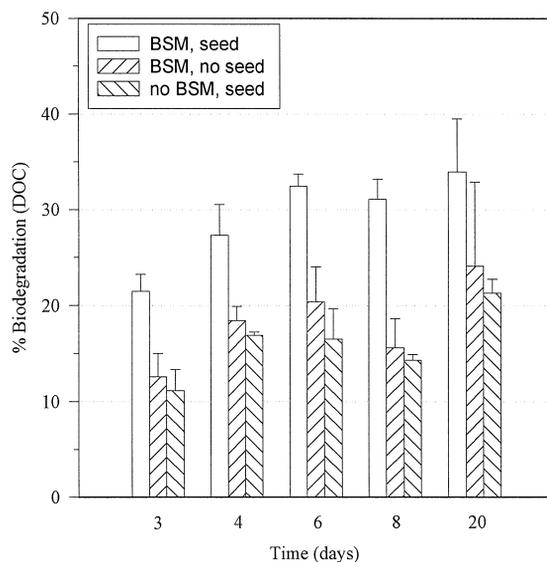
Fig. 1. Effects of nutrients and inoculation on biodegradation (% biodegradation based on DOC) of PPI wastewater.

$21.3 \pm 1.5\%$ (20 days). The % biodegradation in treatment a are significantly higher than that in treatment b at all the sampling time ($p < 0.05$, Fisher's LSD). The mean difference is $13.2 \pm 1.3\%$, suggesting that the wastewater is nutrient limiting and the addition of nutrients (N, P and other minerals) significantly enhanced the biodegradation.

Fig. 1 also shows the effects of inoculation on the biodegradation of PPI wastewater. Similar to the nutrient effects, the effects of inoculation can be seen by comparing treatment a (BSM, seed) with treatment c (BSM, no seed). The biodegradation with nutrient amendments but without inoculation (treatment c) was between $12.6 \pm 2.4\%$ (3 days) and $24.1 \pm 8.8\%$ (20 days), which are significantly lower than the biodegradation under both nutrient amendments and inoculation (treatment a) ($p < 0.05$, Fisher's LSD). The mean difference between these 2 treatments is $11.0 \pm 1.3\%$. This suggests that, with nutrient supplements in the medium, inoculation with acclimated seed will also significantly enhance the biodegradation of PPI wastewater.

Fig. 2 shows the effects of nutrients (BSM) and acclimated seed on the growth of microorganisms. Microbial growth was examined by measuring the protein content in the medium. It was noted that the protein content reached a maximum after 4–6 days of growth, followed by a small decline in the protein content. This decline could be due to the endogenous respiration phase of microorganisms. The protein content after 4 days was in the range of 118.4–145.8, 34.3–43.1, and 63.2–99.1 mg l^{-1} for treatment a (BSM, seed), b (no BSM, seed), and c (BSM, no seed), respectively. The mean difference in the protein content was 76.4 ± 13.5 mg l^{-1} between treatment a and b, and 49.0 ± 6.7 mg l^{-1} between treatment a and c. The differences are statistically significant at a level of 5% (Fisher's LSD), indicating that both the addition of mineral nutrients
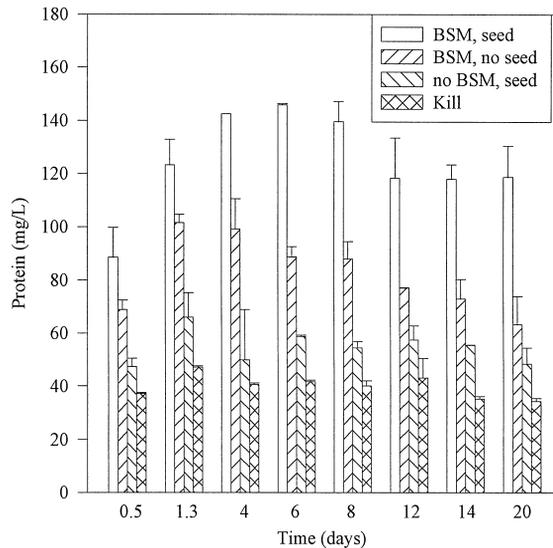
Fig. 2. Effects of nutrients and inoculation on microbial growth (protein content) in PPI wastewater.

and inoculation to the PPI wastewater significantly increased the microbial growth as measured by protein content.

Data in both Figs. 1 and 2 showed that the % biodegradation and the protein content was consistently higher for treatment c (nutrient only) than treatment b (seed only). This may suggest that nutrient amendment is more critical for the biodegradation enhancement. Data shown in Figs. 1 and 2 was obtained when the ratio of PPI wastewater to the media was 80% (v:v). Microbial growth in the PPI wastewater was also tested at a wide range of dilution ratios (i.e. 10%–90%) in order to examine any toxicity of chemicals in the PPI wastewater. This is of concern for the success of bioremediation since dilution will ease the problem if any toxicity exists. The wastewater were inoculated with acclimated seeds and provided with mineral nutrients. All the growth curves were characterized by slow growth rates over time for about 20 days [32]. The gradual increase in microbial growth over time indicated that the acclimated microorganisms are capable of utilizing some organics originally present in the PPI wastewater, but the degradation is very slow. This is understandable since most of the organics in the wastewater (as shown in Table 1) are not readily biodegradable. However, PPI wastewater at a wide range of dilution ratios did not present any apparent toxic effects. In addition, data in Figs. 1 and 2 also showed that the overall percentage biodegradation was low in the time span of the experiments; enhanced biodegradation is therefore necessary for bioremediation to be a feasible and effective alternative on site application.

3.1.2. Effects of surfactant addition

Fig. 3a shows the microbial growth in the PPI wastewater with the addition of different amount of surfactants. A mixture of 4 surfactants was used, including 2 anionic surfactants (sodium dodecylsulfate SDS and sodium dodecylbenzene sulfonate SDBS)
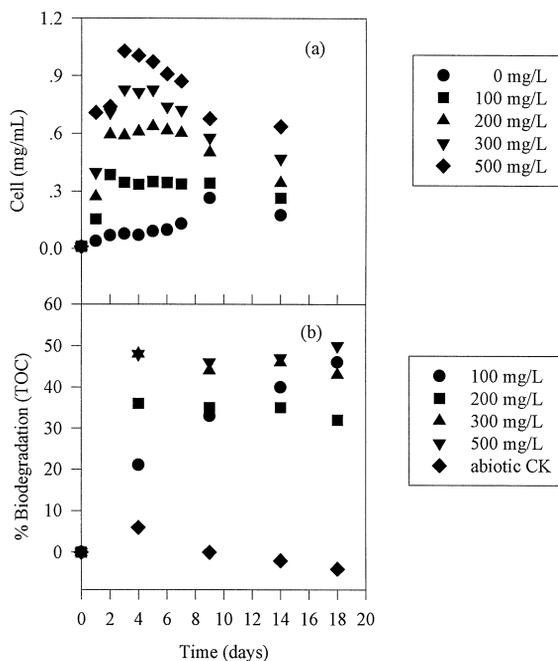
Fig. 3. Biodegradation of PPI wastewater as affected by mixed surfactants at various concentrations: (a) microbial growth and (b) % biodegradation (TOC). Note the concentrations shown are for each surfactant.

and 2 nonionic surfactants (Witconol SN-90 and Tergitol 15-S-12). These surfactants were selected because they have potential use in soil washing [32]. The use of mixed surfactants was recommended from an earlier soil washing experiment, which demonstrated that a blend of anionic and nonionic surfactants removed contaminants more effectively than anionic or nonionic surfactant alone [33]. The concentration of each surfactant was between 100 and 500 mg l^{-1}, which is in the range of 1 to 3 times of the CMCs of the surfactants selected. As shown in Fig. 3a, microbial growth was increased as surfactant concentrations increased. Increased growth was observed when the concentration was as high as 2000 mg l^{-1} (i.e. 500 mg l^{-1} for each surfactant). No inhibitory effects were observed even at such high concentrations of surfactants. These results imply that surfactants at the test concentration are not toxic to microorganisms. On the contrary, the results showed that selected surfactants could serve as a readily available carbon source, thus increasing the biomass in the medium.

Fig. 3b shows the overall percent biodegradation of the mixture of PPI wastewater with different amount of surfactants added. It is noted that biodegradation on day 4 was 21%, 36%, 48%, and 48% when initial concentration of the individual surfactants was 100, 200, 300 and 500 mg l^{-1}, respectively. The increased TOC reduction with increased initial surfactant concentrations at an earlier stage of microbial growth (i.e., 4 days) could be due to the promoted population of microorganisms in the medium (see Fig. 3a), because more surfactants will be utilized as a readily available carbon source to

sustain microbial growth. Fig. 3b also showed that biodegradation reached a maximum after 4–6 days, especially when initial surfactant concentrations are high. This could be the limited mineral nutrients in the medium due to higher concentration of surfactant consumption.

3.2. Biodegradation of surfactant-laden PPI wastewater

3.2.1. Effects of nutrients

An anionic surfactant SDS and a nonionic surfactant Witconol SN-90 were selected and added separately to the wastewater as the surfactant-laden wastewater. The effects of nutrient were then determined by comparing the difference in cell mass, biodegradation, primary biodegradation, and foam degradation between 2 treatments: treatment e (with BSM) and treatment f (without BSM). The results are shown in Fig. 4 for Witconol and Fig. 5 for SDS, respectively. Primary biodegradation refers to the structure of the surfactant was altered such that the basic physical and chemical properties as measured by specific analytical procedures (CTAS for nonionic surfactants, or MBAS for anionic surfactants) are changed [34]. Foam degradation refers to the foam volume reduction for a given volume of sample taken in the course of biodegradation.

As shown in Fig. 4, nutrient amendment significantly enhanced the microbial growth (mg cell ml^{-1}), % biodegradation (DOC), primary biodegradation (CTAS in mg l^{-1}), and foam degradation ($p < 0.05$). Without nutrients, significant primary biodegradation was observed for Witconol at 2500 mg l^{-1} (Fig. 4c), however, no significant changes in biomass, DOC and foam volume were noted (Fig. 4a, b and d). This suggested that the surfactant underwent some structural breakdown into smaller molecules, but the degradation was not enough to maintain significant growth of microorganisms and DOC
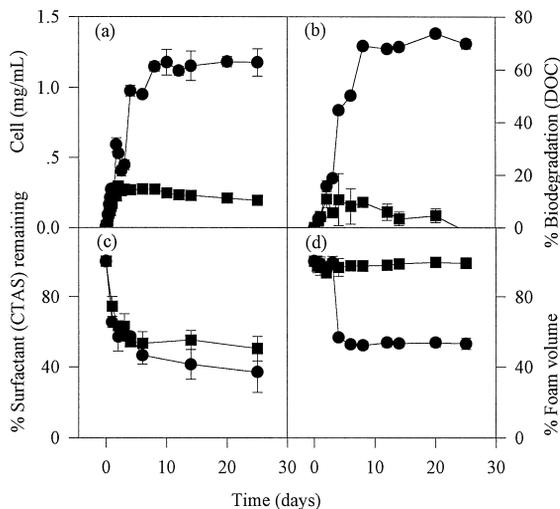

Fig. 4. Effects of nutrients on the biodegradation of Witconol-PPI wastewater mixture: (a) microbial growth, (b) % biodegradation (TOC), (c) % surfactant (CTAS) remaining and (d) % foam volume [$C_0 = 2500$ mg l^{-1} for Witconol; ● with BSM (treatment e); ■ without BSM (treatment f)].
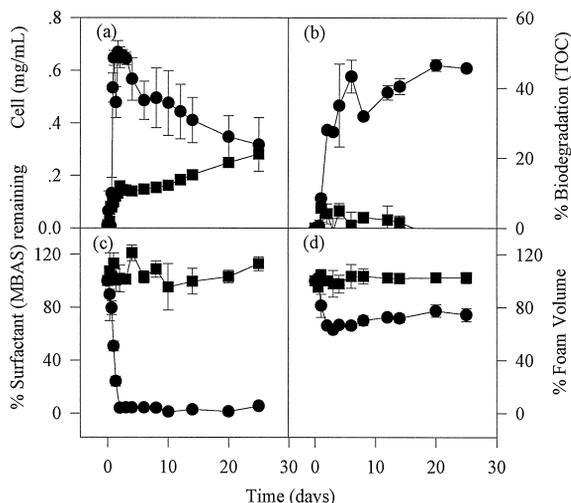
Fig. 5. Effects of nutrients on the biodegradation of SDS-PPI wastewater mixture: (a) microbial growth, (b) % biodegradation (TOC), (c) % surfactant (MBAS) remaining and (d) % foam volume [$C_0 = 2500$ mg l^{-1} for SDS (treatment e); ● with BSM; ■ without BSM (treatment f)].

reductions. It is also noted that, with nutrient supplements, microbial growth reached a logarithmic phase within the first 4 days. Correspondingly, marked increases in % biodegradation and foam degradation were observed from day 3 to day 4. The biodegradation based on DOC were $18.8 \pm 0.4\%$ and $44.6 \pm 1.3\%$ on day 3 and day 4, respectively; while the relative foam volumes were $99.2 \pm 3.6\%$ and $56.7 \pm 1.6\%$ on day 3 and day 4, respectively. Unlike the cell growth, biodegradation and foam degradation, significant primary biodegradation occurred within the first 24 h (Fig. 4c), indicating that Witconol undergo its structural breakdown before an appreciable mineralization and the loss of foamability occurs. With nutrient amendment, foam degradation reached a plateau after 4 days with an average of $53.7 \pm 0.5\%$, suggesting that no further surfactant degradation occurred thereafter. However, microbial growth and biodegradation continued till about 8 days, indicating contaminants other than surfactants in the wastewater were further biodegraded. At the plateau of curves, cell mass maintained an average of 1.16 ± 0.01 mg l^{-1} at the stationary phase and DOC reduction reached its maximum of $69.8 \pm 1.0\%$.

Similar results for surfactant SDS were plotted in Fig. 5. Again, nutrient amendment significantly enhanced bacterial growth, % biodegradation, primary biodegradation, and foam degradation ($p < 0.05$). With nutrient limited treatment, no primary biodegradation and foam degradation was occurred, although a small fraction of microbial growth and biodegradation was observed. With nutrient amendment, primary biodegradation of SDS was almost complete within 2 days, while the biodegradation (% TOC reduction) and foam degradation (% remaining) reached an average of $41.1 \pm 2.1\%$ and $72.0 \pm 1.5\%$, respectively. It was noted that a larger variation existed for the cell mass measurement, possibly due to the interference from the precipitate formed from the reaction between SDS and divalent metals (Ca^{2+}, Mg^{2+}) in the medium.

3.2.2. Biodegradation of combined vs. mixture

The difference in biodegradation between the 'combined' (surfactant or the wastewater separately serving as the only carbon source) and the surfactant–wastewater 'mixture' indicates the comprehensive effects due to interactions between surfactants, microorganisms and contaminants. For surfactant Witconol, the values of DOC $(mg 1^{-1})$ for both 'combined' and 'mixture' were plotted in Fig. 6a, while the biodegradation (% DOC remaining) for these 2 treatments was plotted in Fig. 6b. The percent biodegradation was calculated using Eqs. (1) and (2) for 'mixture' and 'combined', respectively. Similar data for surfactant SDS was plotted in Fig. 7a and b, respectively. However, in the case of surfactant SDS, TOC values were used for the calculation of % biodegradation for the reason as discussed previously.

As shown in Fig. 6a, 2 treatments started with equal values of DOC. After 4 days, however, a marked difference was observed between the combined DOC values and the DOC values of the mixture. The DOCs of the mixture are consistently lower than those of the combined. A nonparametric statistical method using Wilcoxon Rank Sum test showed the difference was significant ($p < 0.05$). It was noted that biodegradation
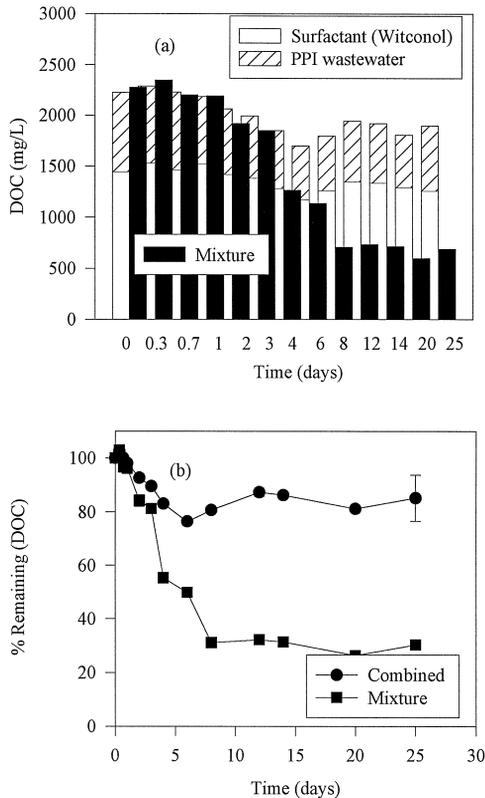

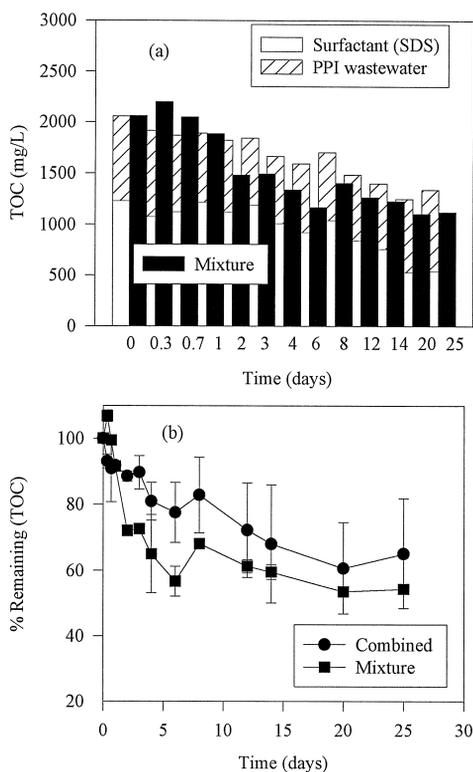Fig. 6. Biodegradation of combined vs. the mixture (Surfactant = Witconol): (a) DOC $(mg 1^{-1})$ and (b) % remaining (DOC).

Fig. 7. Biodegradation of combined vs. the mixture (Surfactant = SDS): (a) TOC $(mg l^{-1})$ and (b) % remaining (TOC).

reached a plateau after 6–8 days. The average biodegradation (% DOC remaining) at this plateau was $82.9 \pm 1.7\%$ for the combined, and $33.5 \pm 3.4\%$ for the mixture. The difference of 49.4% indicates that, by mixing surfactant Witconol and PPI wastewater together, there is a 49.4% enhancement.

Similar data analyses were performed for surfactant SDS. Although the difference between the combined and the mixture appears to be much smaller than that of Witconol, statistical analysis with Wilcoxon Rank Sum test again showed the significance ($p < 0.05$). The average biodegradation (% TOC remaining) between 6 and 25 days was $71.1 \pm 3.34\%$ for the combined and $58.9 \pm 2.2\%$ for the mixture. The enhancement based on TOC reduction was therefore 13.1%.

The biodegradation enhancement suggested that mutually beneficial effects existed between the surfactants and contaminants in the mixture during biodegradation. The results also showed the absence of toxicity to microorganisms degrading surfactants and organic compounds. Moreover, for up to 50% enhancement in biodegradation of the Witconol–PPI wastewater mixture, it is reasonable to assume that the presence of nonionic surfactant (Witconol) greatly enhanced the biodegradation of contaminants in the PPI wastewater (see also the discussion below). Several mechanisms existed for the

surfactant enhanced biodegradation. (1) The enhancement by surfactant could be due to the promoted microbial growth (Fig. 3a, since surfactant can be utilized as a readily available carbon and energy source. (2) Surfactant enhancement could also be the result of co-metabolism between surfactants and contaminants, since the concentration of some of the contaminants could be below the threshold to sustain significant microbial growth. (3) The presence of surfactant will not only stimulate bacterial growth, but also induces enzymes (proteins) that may be needed to further break down contaminants. The protein data from this study is a supporting evidence, since the same pattern of protein contents was observed between the combined and the mixture [32]. (4) Surfactant enhancement could also be the enhanced solubility and/or the greater bioavailability of HOCs to the microorganisms.

One may argue that the synergistic effects might be the enhanced surfactant biodegradation due to some compounds in the wastewater. For instance, an earlier study showed that microorganisms grown on petroleum hydrocarbons will produce biosurfactants [35]. Some nutritional components in the wastewater may be also attributed to this. However, it was proved to be very unlikely due to the nature of the PPI wastewater. A study was conducted therefore to test the biodegradation of surfactants with the addition of different amount of PPI wastewater, results showed that the enhanced biodegradation of surfactant by components in the PPI wastewater was insignificant [32].

4. Conclusions and implications

Laboratory shaker-flask experiments were conducted to study the biodegradation of wastewater collected from a local Superfund site, which contains a variety of hydrocarbon contaminants. Potential enhancement of biodegradation was studied for the wastewater with the amendment of surfactant and nutrients, and the inoculation of seed. The following conclusions are drawn from this study.

· When PPI wastewater served as the only carbon source, biodegradation with acclimated seeds was characterized by a slow process with a low percentage of TOC degraded. PPI wastewater is nutrient limited, and addition of nutrient media increased TOC reduction by 13%, while inoculation with acclimated seeds also improved the percentage of TOC removal by 11%. Addition of surfactants to the PPI wastewater as a readily available carbon source promoted microbial growth and accelerated the biodegradation process.

· For the mixture of SDS and PPI wastewater, nutrient addition significantly increased microbial growth and biodegradation of the mixture, and primary and foam degradation of the surfactant. No significant degradation occurred unless external nutrients were provided.

· For the mixture of Witconol and PPI wastewater, nutrient addition significantly increased microbial growth, biodegradation and foam degradation. Primary biodegradation of the surfactant occurred in the absence of nutrients, and the addition of nutrients only slightly promoted primary biodegradation.

· A synergistic effect on biodegradation was found when comparing the biodegradation of the 'mixture' (surfactant and PPI wastewater) with that of 'combined' (where

surfactant or PPI wastewater separately served as the only substrate). The enhancements based on TOC reduction were 13% for the mixture of PPI wastewater with SDS, and 49% for the mixture of PPI wastewater with Witconol.

· Mixed surfactants at a total concentration as high as 2000 mg l^{-1} showed no inhibitory effects on the microbial growth in the wastewater. Also, organic contaminants encountered in the wastewater had no apparent toxic effects on the surfactant-degrading microorganisms, indicating that microorganisms have been well adapted to the toxic chemicals present, or they are below toxic levels to the microorganisms [32].

The results indicated that the intrinsic biodegradation of the PPI wastewater is slow, which may be attributed to the lack of nutrients and the recalcitrant nature of the chlorinated hydrocarbons in the PPI wastewater. Results also indicated that enhanced biodegradation could be achieved through the amendment of both mineral nutrients and carbon source (surfactant, etc.). The nutrient enhancement were observed for PPI wastewater and surfactant-laden PPI wastewater as well, indicating that supplements with mineral nutrients are required for optimal degradation to occur even with the presence of sufficient carbon source. The results on surfactant augmented biodegradation are very encouraging. However, this study did not determine which organic chemicals were biodegraded during the surfactant enhancement. We surmise that lower chlorinated hydrocarbons may be co-metabolized through surfactant addition. The co-metabolism potential of lower chlorinated aliphatic hydrocarbon with the presence of carbon source in an aerobic environment was demonstrated in a recent study [36]. For highly chlorinated hydrocarbons however, dechlorination takes much longer time and prefers anaerobic condition. Surfactant enhancement is an important area for additional research. Further experiments will be directed to monitor and verify individual contaminants during the course of biodegradation using GC/MS analysis.

Biodegradation with multiple substrates (including surfactants) is of significance since this is what is occurring in the real environment. At high initial concentrations with combinations of carbon sources, a synergistic effect results in increased specific growth rates compared to growth with the single substrates [37]. Similar results were also reported by Engli et al. [38], their results showed that the residual concentrations of individual compounds were consistently lower during mixed substrate growth than during growth with the single substrates. The increased microbial growth and biodegradation in the mixture as compared to that in the combined from this study warrant further research. Future studies are needed to clarify the mechanisms involved in the biodegradation enhancement with surfactants. Several researchers have addressed this issue on individual contaminants, and their findings were diverse [12,13,39,40]. Surfactants could serve as a readily available carbon source and facilitate the biodegradation process by increasing the microbial population. Surfactants may also increase the aqueous solubility of HOCs, which is usually the limiting step for bacterial utilization. The information currently available on the effects of surfactants on individual compound has limited use, since in the case of actual wastewater containing multiple contaminants, the mechanisms may be several and more complicated.

It can also be concluded that through the use of appropriately selected surfactants in soil washing, bioremediation may be a cost-effective intermediate step between soil washing and subsequent treatments. Enhanced biodegradation of certain compounds

along with the biodegradation of surfactants themselves will increase the overall treatment efficiency while keeping surfactant-induced problems at a minimal level, allowing other unit processes (e.g. air stripping, activated carbon) to be incorporated for further treatment on recalcitrant contaminants. Such recalcitrant contaminants in the waste stream, due to long half-lives, may require non-biological means such as air stripping or activated carbon adsorption that are in common use.

Acknowledgements

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