Effect of additional carbon source on biodegradation of linear alkylbenzene sulfonate by las-utilizing bacteria

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Abstract

Aerobic biodegradation of linear alkylbenzene sulfonate (LAS) by LAS-utilizing bacteria (LUB) in the presence of other sources of carbon (glucose and soluble starch) was examined. Biodegradation of LAS was monitored as primary degradation in terms of half-life ($t_{1/2}$) of the surfactant. Biodegradation of LAS by the individual LUB was slower in the presence of Glucose. Biodegradation of the surfactant by the various consortia of LUB was slower in the presence of the carbon sources: $t_{1/2}$ increased to 3 days. The rates of biodegradation by the consortia can be ranked as: four-membered ($t_{1/2}=9$ days) > three-membered ($t_{1/2}=9$ to 13 days) > two-membered consortia ($t_{1/2}=10$ to 15 days). Generally, degradation in the presence of the carbon sources was faster with the consortia than the individual species. Degradation of the surfactant by the LUB was generally fastest in the absence of additional carbon sources. The possible role of additional carbon sources in persistence of surfactant in water bodies and the application of the observation in management of LAS-containing-effluent is suggested.

Introduction

Before 1945, synthetic detergents were virtually unknown, but gained public attention within a few years due to water pollution problems. Alkylbenzene sulfonate (ABS) replaced soap as domestic and industrial cleaning agents because of their cheapness, greater efficiency and did not cause precipitation of calcium salts in areas with hard water.¹ ABS (hard detergent) did not readily breakdown in sewage treatment processes giving rise to environmental complications: problems of toxicity to aquatic organisms and aesthetics-undesirable presence of foam scum on water.² ABS was subsequently banned and gradually replaced in the 1960s by linear alkylbenzene sulfonate (LAS), which is readily biodegradable.³

The degradation of surfactant is of great

interest, and remains a factor in the acceptability or rejection of surfactants. Aerobic biodegradation of LAS can be divided into primary degradation and ultimate degradation. Primary degradation involves the disappearance of the parent molecule and loss of interfacial activity. It is initiated by alkylsulfatase whose presence is suggested to be synonymous with ability to accomplish mineralization of LAS.⁴ Studies have shown that primary degradation occurs during the initial reactions in the metabolic pathway. Primary degradation is transformation induced by microorganisms resulting in the production of short chain homologues: sulphophenyl carboxylates (SPCs).⁵

Ultimate degradation is the cleavage of the aromatic ring and complete conversion of LAS and SPCs to water, CO₂, inorganic sulphates and biomass. Ultimate degradation of LAS involves the action of two or three tiers of bacteria.⁶ The first tier organisms possess enzymes for ω - and β -oxidation of the alkyl chains. The second tier organisms convert the resultant long chain SPCs (lc-SPCs) by further β -oxidation of the alkyl chain, forming short chains SPCs (sc-SPCs). The third tier organisms mineralize the sc-SPCs via 4sulfocatechol and ortho ring cleavage.^{3,5} The opening of the aromatic ring is the rate-limiting step for ultimate degradation of LAS.⁷

Most studies on biodegradation of LAS have considered situation in which the surfactant is present as sole carbon source. This is a rare occurrence because the aquatic environment, in particular serves as receptacle for a large number of pollutants; no single pollutant exists alone in the environment. Some materials have simple structures that make them more prone to microbial attack than other more complex molecules. Also some organisms have preference for some types of carbon substrates. In this study, the biodegradation of LAS in the presence of other utilizable carbon source is examined. Individual and consortia bacteria were examined in their ability to degrade the surfactant in the presence of glucose and soluble starch as additional sources of carbon. Degradation of LAS was monitored as half-life ($t_{1/2}$), which is the time it takes for microbes to breakdown half the amount of a chemical in water.

Materials and Methods

Selection of organisms

Alcaligenes odorans, *Citrobacter diversus*, *Micrococcus luteus* and *Pseudomonas putida* previously isolated from detergent-effluent polluted stream⁸ were selected from among the LAS-tolerant bacteria. The bacterial isolates

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were screened for ability to degrade LAS by plating them separately on Mineral salt medium containing LAS as sole carbon source. Organisms with degradative ability were then selected and standardized as 0.5 McFarland standard (10⁸ cell/mL) and were used as inoculum in degradation study.

Degradation studies

LAS mineral broth was prepared by dissolving anhydrous LAS (1% w/v) in mineral salt medium⁹ glucose-supplemented and soluble starch-supplemented LAS broths were then prepared by dissolving 1% (w/v) of the carbon source (glucose and starch) in the broth. Flasks containing 300 mL of medium was inoculated with 10 mL broth culture of standardized selected organisms as individual pure cultures and various consortia of two, three and four members. The inoculated media were incubated on an orbital shaker (100 rpm) at room temperature (28±2°C).

Aliquots (10 mL) of the resulting cultures were withdrawn daily and the concentration of surfactant determined using the Hyamine titration method.¹⁰ Biodegradation was monitored daily as primary degradation in terms of half-life of the surfactant. The half-life is indicated by 50% reduction in initial concentration of surfactant. An un-inoculated medium treated in the same way served as control. Data obtained were analyzed statistically using the ANOVA.

Results

The effect of the additional carbon sources on the degradation of the surfactant by the organisms is shown on Figures 1-3. It shows that the rate of degradation was fastest in all cases when there was no other source of car-

bon. The presence of glucose increased $t_{1/2}$ by 3 days: *Pseudomonas putida*; 2 days: *Citrobacter diversus*; 3 day: *Micrococcus luteus* and 3 days: *Alcaligenes odorans*. Soluble starch increased $t_{1/2}$ by 2 days: *Pseudomonas putida*; 1 day: *Citrobacter diversus*; 2 day: *Micrococcus luteus* and 1 days: *Alcaligenes odorans* (Figure 1). In the case of the two-membered consortia of organisms, the presence of the additional carbon sources increased $t_{1/2}$ by 2 days in all cases except for the consortium of *Alcaligenes odorans* and *Citrobacter diversus*, in which case the presence of starch increased $t_{1/2}$ by 1 day (Figure 2).

Among the three-membered consortia, the presence of glucose increased $t_{1/2}$ by between 4 days (*Citrobacter diversus* *M. luteus* and *P. putida*) and 2 day (*A. odorans* *Citrobacter diversus* and *P. putida*; *A. odorans*, *M. luteus* and *P. putida*). In the case of soluble starch; $t_{1/2}$ increased by between 3 days (*A. odorans* *Citrobacter diversus* and *P. putida*; *Citrobacter diversus* *M. luteus* and *P. putida*) and 2 days (*A. odorans*, *C. diversus* and *M. luteus*; *A. odorans*, *M. luteus* and *P. putida*). The carbon sources increased $t_{1/2}$ by 2 days each in the case of the four-membered consortium (Figure 3). The rates of degradation by the consortia can be ranked as: four-membered ($t_{1/2}$ = 8 days) > three-membered ($t_{1/2}$ = 8-13 days) > two-membered ($t_{1/2}$ = 10-15 days) consortia. Generally, biodegradation of LAS was slowest in the presence of glucose and fastest when LAS was sole carbon sources were absent. There was no significant difference in the rates ($t_{1/2}$) of degradation by the selected organisms and their ($P < 0.05$).

Discussion

The difference in rate of degradation by pure cultures and consortia of the organisms in the presence of additional carbon sources suggest the influence of availability of alternative carbon source. This is attributable to the preferential selection of easily utilizable substrates by microorganisms over other substrates.¹¹ The inhibitory effect of carbon sources on biodegradation of styrene has been reported.^{12,13} The effect of the presence of additional carbon sources observed in this study is consistent with the higher anaerobic degradation of LAS in the absence of additional sources of carbon that has been observed.¹⁴ Similarly, sludge acclimatized to the degradation of phenol showed an initial preference for easily degradable co-substrates such as glucose with only a slow concomitant assimilation of phenol.¹⁵ However, assimilation of phenol increased rapidly after the co-substrates were depleted and phenol was preferentially taken in the presence of a less easily degradable co-

substrate.¹⁶

The observed effect partly explains the persistence of the surfactant in water bodies which was observed by Eniola and Olayemi.¹⁷ The water bodies usually serve as receptacle for a large number of pollutants, many of which are simpler in structure compared to the LAS. The presence of more readily utilizable molecules could create a situation in which the surfactant is only co-metabolized while other molecules are used as source of carbon. Co-metabolism is defined as the transforma-

tion of a non-growth substrate in the obligate presence of a growth substrate or other transformable substance.¹⁸ Co-metabolism could result in intermediate products that are recalcitrant or more toxic than LAS.¹⁹

The slower rate of degradation of the surfactant in the presence of glucose is because glucose is a substrate that is readily used by most organisms. It can be readily taken into the cell by the group translocation system as against the LAS that would need to be broken first. Rate regulation would be informed by the bal-

Ps. Putida, *Pseudomonas putida*; *C. Diversus*, *Citrobacter diversus*; *MI luteus*, *Micrococcus luteus*; *Al. odorans*, *Alcaligenes odorans*.

AC, *A. odorans* and *C. diversus*; AM, *A. odorans* *M. luteus*; AP, *Alcaligenes odorans* *P. putida*; CM, *Citrobacter diversus* *M. luteus*; CP *Citrobacter diversus* *P. putida*; MP *M. luteus* *P. putida*.

ACM, *A. odorans*, *C. diversus* and *M. luteus*; ACP, *A. odorans* *Citrobacter diversus* and *P. putida*; AMP, *A. odorans*, *M. luteus* and *P. putida*; CMP, *Citrobacter diversus* *M. luteus* and *P. putida*; ACMP, *A. odorans*, *C. diversus*, *M. luteus* and *P. putida*.

Figure 1. Biodegradation of LAS by individual LAS-utilizing bacteria in the presence of glucose and starch.

Figure 2. Biodegradation of LAS by two-membered consortia of LAS-utilizing bacteria in the presence of glucose and starch.

Figure 3. Biodegradation of LAS by consortia of LAS-utilizing bacteria in the presence of glucose and starch.

ance of equilibrium between substrate and product; hence the presence of glucose is likely to tilt the reaction away from the breakdown of LAS. The transformation products of LAS are feed into the central metabolic pathway (Krebs cycle and glyoxylate cycle) present in bacteria.²⁰

The presence of additional sources of carbon could serve, on the long run, as a means of building up biomass for eventual degradation of the LAS. Yediler *et al.*²¹ reported that a direct correlation between the rate of primary degradation and the initial bacterial population. Dentel *et al.*²² similarly associated the complete mineralization of LAS to the number of organisms present. This is perhaps part of the reason why there is no statistical difference ($P < 0.05$) in the rates of degradation of the surfactant by pure cultures and their consortia.

Conclusions

Generally, biodegradation of LAS was slowest in the presence of glucose and fastest when LAS was sole carbon sources were absent. The presence of the additional carbon source slowed down the degradation of LAS by the organisms. The consortia also degraded LAS at a faster rate than the individual pure culture. This study provides information that can be useful in the operating Waste treatment plant (WTP) for LAS-containing effluents. For such WTP, it is important to use consortia of organisms; in addition wastes that contain easily degradable carbon sources should not be pooled with LAS-containing wastes.

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