

Effect of pH on Solubilization of Phenanthrene and Rhamnolipid Morphology

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PURPOSE

The effect of pH on the solubility of rhamnolipid biosurfactants in aqueous were investigated to evaluate their potential in enhancing the solubilization of phenanthrene. In previous experiments, the order of increasing solubility enhancement of phenanthrene was pH 8 < pH 7 < pH 6 < pH 4 < pH 5. Phenanthrene solubilization experiments in an aqueous system were performed. As the pH was increased from 5 to 7, the apparent solubility of phenanthrene decreased. To confirm this explanation for the different solubilizing capacity results from the structural change of biosurfactant aggregate, cryo-transmission electro microscopy was employed. Micrographs showed the rhamnolipid morphology changed.

MATERIALS and METHODS

Sample preparation. Samples of 26 mM rhamnolipid were adjusted to the appropriate pH using 1 N NaOH or 0.1 N HCl solution, and filtered with a 0.45 m filter. The sample pH remained stable over the duration of the experiment. *Grid preparation.* A glass slide was coated with a film of formvar-chloroform-glycerol solution and then held over steam to produce a thin web of "holey" plastic. The film was placed on one side of a cryo grid, dried, and then carbon coated. A drop of sample (less than 7 l) was placed on the grid and drawn into the holes by slightly running the back of the grid down a piece of filter paper. The grid was immediately plunged into a bath of liquid ethane and transferred to a liquid nitrogen bath. The samples were stored in a GATAN model 630 cryotransfer (Gatan, Inc., Warrendale, PA) under liquid nitrogen, at approximately -185°C (Champion et al., 1995). *Cryo-transmission electron microscopy.* Samples were viewed and photographed at approximately -170°C using a Tecnai 12 electron microscope (Philips, Eindhovenm Netherlands) at 120 kV, and the images were acquired with a Multiscan 600W CCD camera (Gatan, Inc., Warrendale, PA).

RESULTS

The cryo-TEM micrographs showed that large lamellar sheets dominated at pH 4, with the presence of tubular and irregular bilayered structures. At pH 5, large lamellar sheets predominated, with the existence of vesicles. Multilamellar vesicles and large unilamellar were also frequently found. Generally, large structures were dominant at pH 4 and 5. The vesicles were also observed at pH 6, but bilayered vesicles rarely existed. Compared to the structures at pH 5, the number of small vesicles increased and spherical micelles were also seen. At pH 7, the presence of small micellar structures were found, but no vesicles were observed.

CONCLUSION

The pH variation can significantly affect the rhamnolipid morphology, and these structural changes induce variations in the phenanthrene solubility. The highest solubility was detected in pH 5 for the rhamnolipid solution in the aqueous system, indicating that lamellar or large vesicles are more effective for phenanthrene solubilization, as based on the cryo-TEM micrographs. Therefore, it is clear that anionic surfactants can be more powerful for contaminant solubility when solution pH control is used.