

A comparison of five extraction methods for extracellular polymeric substances (EPS) from biofilm by using three-dimensional excitation-emission matrix (3DEEM) fluorescence spectroscopy

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Abstract

Two physical methods (centrifugation and ultrasonication) and 3 chemical methods (extraction with EDTA, extraction with formaldehyde, and extraction with formaldehyde plus NaOH) for extraction of EPS from alga-bacteria biofilm were assessed. Pretreatment with ultrasound at low intensity doubled the EPS yield without significant modification of the composition of EPS. Extraction with EDTA or extraction with formaldehyde plus NaOH increased yield by about 1 order of magnitude compared with other methods. However, the protein and polysaccharide content in EPS prepared with EDTA or formaldehyde plus NaOH were low. Two fluorescence peaks belonging to protein-like peaks and 2 fluorescence peaks belonging to humic acid-like substances were found in 3DEEM fluorescence spectra of all the EPS samples prepared using different methods. Fulvic-like fluorescence was detected only in the EPS extracted with formaldehyde plus NaOH. Location of, and fluorescence intensity at, each peak were clearly affected by the extraction methods. Dialysis was also found to be an important factor influencing the yield, composition and fluorescence characteristics of EPS.

Keywords: biofilm, extracellular polymeric substances, 3DEEM fluorescence spectroscopy