

The effect of dissolved humic substances on the growth and alkaline phosphatase of microalgae

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Dissolved humic substances (DHS) coming from terrestrial, wetland, and littoral plants often comprise the major portion of dissolved organic matter in aquatic environment. These compounds can combine proteins and affect the physiology of algae or inhibit enzyme activities. Alkaline phosphatase (AP), as an enzyme located at the cell surface, is susceptible to the inhibition by humic substances. The effect of humic substances were evaluated by measuring the inhibition of AP and the effect on the algal growth. Axenic cultures of *Anabaena flos-aquae*, *Microcystis aeruginosa*, *Selenastrum capricornutum*, *Scenedesmus brasiliensis*, and *Nitzschia palea* were employed as the test algae. DHS from two sources were used to analyze the inhibition, standard humic and fulvic acids from Suwanee River and the humic and fulvic acids purified from decomposed macrophyte *Typha latifolia*.

AP showed large variation with species in the substrate affinity, K_m , for the artificial substrate, methyl umbelliferyl phosphate. K_m was the largest ($>500 \mu\text{M}$) for green algae, *Scenedesmus brasiliensis* and *Selenastrum capricornutum*, $250 \mu\text{M}$ for *Anabaena flos-aquae*, and a diatom, *Nitzschia palea*, showed smallest K_m ($2 \mu\text{M}$). Extracellular free enzyme of *Anabaena flos-aquae* showed smaller K_m ($52 \mu\text{M}$) than did cell-bound enzymes. Purified bovine AP exhibited smaller K_m ($1.7 \mu\text{M}$) than did algal AP.

DHS showed mixed-type noncompetitive inhibition of AP, affecting both K_m and V_{max} . The inhibition constant K_i varied with algal species and the location of enzyme; cell-bound or free extracellular. *A. flos-aquae* and *S. brasiliensis* showed smaller K_i (i.e. more inhibited) than *N. palea* and *S. capricornutum*. Free enzyme showed smaller K_i than cell-bound form. Purified bovine AP showed smaller K_i by one order of magnitude than algal AP.

Contrary to the consistent inhibition of AP, humic substances enhanced or suppressed algal growth. The two cyanobacteria, *A. flos-aquae* and *M. aeruginosa* were suppressed, whereas two green algae, *S. capricornutum* and *S. brasiliensis* were not affected.