A MERCURY BUFFER FOR TOXICITY EXPERIMENTS WITH GREEN ALGAE

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Abstract. Mercury(II) toxicity experiments with green algae are complicated by the fast reduction and evaporation of Hg. A Hg buffer system is described, which considerably stabilizes the Hg(II) concentration in test solutions. The Hg buffer consists of mercury(II) chloride and N-methyliminodiacetic acid (MIDA). Dissociation of Hg-MIDA complex compensates for loss of Hg. With this system experiments were performed with Hg(II) concentrations between 0.02 and 2.0 mg l⁻¹ at temperatures between 15° and 30°C. No effect of MIDA on the growth of the green alga Scenedesmus acutus was detected.

1. Introduction

In our investigations on biological effects of thermal pollution we studied the effect of temperature on toxicity of Hg(II) in the green alga Scenedesmus acutus. During the experiments concerned, which were performed in batch cultures for up to two weeks, unacceptable losses of Hg from the test solutions were repeatedly recorded. Similar observations were made by others (Newton and Ellis, 1974). Four causes for loss of Hg from solutions are given: (a) chemical reduction of Hg(II) to Hg(I), followed by disproportioning of the Hg(I) to Hg(II) and Hg(0) which evaporates rapidly; (b) metabolic activity of micro-organisms, comprising either reduction of Hg(II) to elemental Hg or formation of organic Hg compounds which leads to loss of Hg, resulting from evaporation of these products; (c) adsorption of Hg on the wall of the solution container; (d) leakage of Hg through the wall of the solution container.

In the concentration range used in our experiments (up to 10⁻⁵ M) all these causes for loss of Hg have played a part. The fact that growing cultures of algae had greater Hg loss than the same medium without algae, might indicate that in our experiments the reduction of Hg(II) by algal reducing agents gave rise to losses of Hg. Ben-Bassat et al. (1972) made similar observations using the green alga Chlorella pyrenoidosa 211/8h. Our assumption was confirmed by measuring the redox potential of the medium without and with algae; the latter had a significantly lower redox potential.

In addition to the described causes for Hg loss there is a decrease of the 'free' Hg(II) in cultures because of binding by algal cellwalls (unpublished observations). Yet it was necessary to keep the Hg concentration during our toxicity experiments constant. In natural waters the concentrations of metals are stabilized by the buffering capacity of dissolved, suspended and precipitated material with metal-binding properties. This observation prompted us to select a Hg chelating agent as a
Hg buffer in our culture medium. The chelator needed the following characteristics: (1) the stability constants of the Hg(II) complex should guarantee sufficient buffering capacity in the concentration range of our experiments (0.02 to 2 mg l\(^{-1}\)); (2) the chelator has to be neither an inhibitor of nor a nutrient to the algae; (3) the stability constants of the Hg complexes have to be known for different temperatures, to enable us to perform comparable experiments at those temperatures. On the basis of data by Chaberek and Martell (1959) we tested some chelators and selected N-methyl-iminodiacetic acid (MIDA). This compound has the above mentioned characteristics as will be shown in the experimental part.

Schwarzenbach et al. (1955) described a variety of possible complexes between Hg(II), MIDA, H and OH ions. The complexes and reaction equations that are important under our experimental conditions are given below; MIDA is given as a dibasic acid H\(_2\)A.

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\begin{align*}
H_2A & \rightleftharpoons HA^- + H^+ \\
HA^- & \rightleftharpoons A^{2-} + H^+ \\
Hg^{++} + A^{2-} & \rightleftharpoons HgA \\
HgA + A^{2-} & \rightleftharpoons HgA_2^{2-}
\end{align*}
\]

Using the dissociation constants of these equations, Chaberek and Martell's (1959) data and our measurement, quantities of Hg(II) and MIDA can be calculated to give a fixed concentration of 'free' Hg(II) and a large Hg-buffering capacity.

2. Material and Methods

2.1. CULTURE CONDITIONS

*Scenedesmus acutus*, from the collection in our laboratory, was grown asceptically in 50 ml medium in 100 ml round-bottom flasks with a side tube for direct measurement of the optical density of the culture.

Optical density was used as an estimate for algal cell concentration. In some of the experiments the optical density was measured at a wavelength of 430 nm, in others at a wavelength of 750 nm. At 430 nm the chlorophyll of the algae contributes markedly to the optical density of the suspension. At 750 nm, however, the optical density of the cultures is caused by the turbidity of the cell suspension only.

Inocula were taken from logarithmic phase cultures. The flasks were incubated in a Gallenkamp Orbital shaker at 25°C with fluorescent illumination at 24.6 W m\(^{-2}\) (6 lamps of 30 W) in a 16 h light—8 h dark regime. The orbital shaker had a rotating speed of 100 rpm. Air with 1% CO\(_2\) was passed over the medium at a rate of 5 l h\(^{-1}\).

The medium was composed as follows:

- 965 ml demineralized water
- 350 mg KNO\(_3\)