# Influence of U(VI) on the metabolism of plant cells studied by microcalorimetry and TRLFS

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**Uranium(VI) shows a concentration-dependent influence on the metabolic activity of plant cells. With increasing U(VI) concentration, the predominant U(VI) species in medium $R_{red}$ changes from $UO_2HPO_4$(s) to $(UO_2)_3(OH)_5^+$, which may affect the bioavailability of U(VI).**

Knowledge of the radionuclide transfer in the environment including the food chain is the basis for the reliable risk assessment for humans. The interaction of plants with heavy metals impacts their metabolism. For instance, plants segregate metal chelates into the rhizosphere, store metal chelates in vacuoles or synthesize protective metabolites.[1] We studied the interaction of U(VI) with canola cells (*Brassica napus*) focusing on the concentration-dependent impact of U(VI) on the cell metabolism. Isothermal microcalorimetry was used to monitor the metabolic heat flow. The cell viability was studied using the MTT test.[2] The speciation of U(VI) in the nutrient medium was determined by time-resolved laser-induced fluorescence spectroscopy (TRLFS) and thermodynamic modeling to correlate the impact of U(VI) on the cell activity with its speciation.

**EXPERIMENTAL.** Starting from a callus cell culture (P-1113, DSMZ), suspension cultures of *Brassica napus* cells were grown in medium R[3] at room temperature on a gyratory shaker. Isothermal microcalorimetry was performed with a TAM III instrument (Waters GmbH)[4] to continuously monitor the metabolic heat flow produced by the cells in the absence or presence of U(VI) at 25 °C for up to 311 h. Wet cells (0.3 g) were transferred into glass ampoules and 2 mL of medium R with a reduced phosphate concentration of $1.25\times10^{-5}$ mol/L (medium $R_{red}$; pH 5.8) were added. To study the impact of U(VI) on the cell metabolism, U(VI) was added in form of a $UO_2(NO_3)_2$ stock ($[U(VI)]_{final}$: $2\times10^{-5}$–$2\times10^{-4}$ mol/L). After the microcalorimetric experiments, the cell viability was determined by the MTT test.[2] The U(VI) speciation in medium $R_{red}$ was studied by time-resolved laser-induced fluorescence spectroscopy (TRLFS) using a Nd-YAG pumped OPO systems (Newport-Spectra Physics)[5] and calculated by thermodynamic modeling (Geochemist's Workbench).[6]

**RESULTS.** Figure 1 illustrates the metabolic heat flow of the cells at different U(VI) concentrations. It decreases with increasing U(VI) concentration indicating a decrease of the metabolic cell activity. Based on these data the "metabolic endurance", which represents the product of the metabolic power and the total released heat, was derived (inset Fig. 1). The maximum values obtained at different U(VI) concentrations were normalized to the value determined in the absence of U(VI). These data were compared to the cell viability data measured by MTT test, which is based on the activity of mitochondrial and cytosolic dehydrogenases of living cells (Fig. 2). Both the heat flow data and the cell viability data decrease with increasing U(VI) concentration and agree very well. TRLFS results show that $UO_2HPO_4$ (s) dominates the U(VI) speciation at $2\times10^{-5}$ mol/L U(VI) in medium $R_{red}$ (pH 5.8), which is confirmed by thermodynamic modeling. With rising U(VI) concentration, the U(VI) speciation changes continuously and is dominated by U(VI) hydroxo

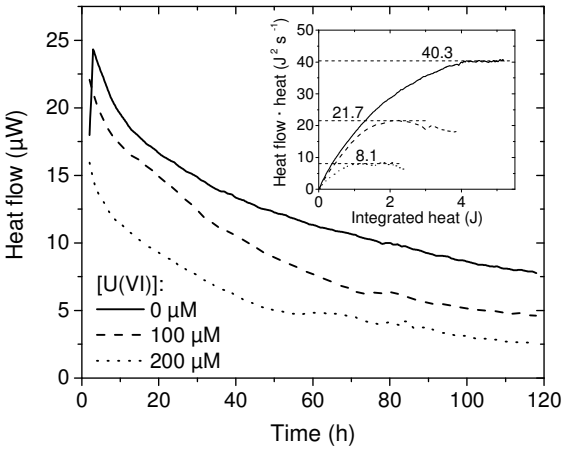


Fig. 1: Heat flow of the cells at different U(VI) concentrations and derivation of the "metabolic endurance" (inset).

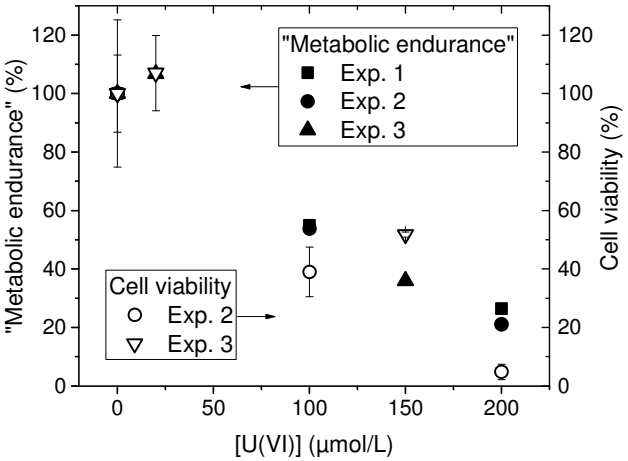


Fig. 2: Comparison of the normalized "metabolic endurance" with the cell viability measured by the MTT test.

complexes. At $[U(VI)] \geq 1\times10^{-4}$ mol/L, the speciation is predominated by the $(UO_2)_3(OH_5)^+$ complex (60–65 % of total U(VI)). In addition to the total amount of U(VI), the decrease of the cell metabolism may be attributed to the change of the U(VI) speciation in solution, which can affect its bioavailability.

Isothermal microcalorimetry is a highly sensitive real-time monitor of the concentration and probably speciation-dependent U(VI) toxicity in *Brassica napus* cells. The metabolic response of the plant cells correlates very well with their mitochondrial activities. This opens further possibilities to study low dose effects of U(VI) using different isotopes in calorimetric experiments.

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