

Experiments were conducted to isolate and identify the intermediates and products of 2,4-dinitrotoluene and 2,6-dinitrotoluene metabolism by *Clostridium acetobutylicum*. Transformation of both dinitrotoluenes initially resulted in the formation of hydroxylaminonitrotoluenes. Subsequent transformation favored the formation of dihydroxylaminotoluenes, with a limited reduction to aminonitrotoluene isomers. In cell cultures, metabolism beyond the level of dihydroxylaminotoluene was not observed. In cell extracts, where activity could be maintained for periods in excess of those in cell cultures, further transformation yielded aminohydroxylaminotoluenes and eventually diaminotoluenes. These findings further demonstrate the potential for hydroxylamines to be significant intermediates of nitroaromatic transformation under anaerobic fermentative conditions. Interestingly, the rearrangement of dihydroxylaminotoluenes was not observed, as was the case in previous studies of 2,4-dihydroxylamino-6-nitrotoluene metabolism (e.g., the dihydroxylamino metabolite of 2,4,6-trinitrotoluene transformation by *C. acetobutylicum*). Dihydroxylaminotoluenes were found to be quite unstable, decomposing rapidly upon exposure to oxygen, complicating the assessment of their fate in remediation processes.