Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), a military high explosive, is becoming an increasingly important pollutant in the US. The cleanup of RDX-contaminated soil and groundwater has been a serious challenge due to its recalcitrance in the environment. This study was conducted to determine the biodegradation kinetics of RDX by crude cell extract of Clostridium acetobutylicum (ATCC 824), and to examine whether this bacterium will carry out reductive transformation pathways similar to the transformation of 2,4,6-trinitrotoluene (TNT), 2,4- and 2,6-dinitrotoluenes (DNTs) we have reported previously. Batch studies on the anaerobic transformation of RDX were conducted in serum bottles with U-ring-14C-RDX. RDX and its transformation products were quantified by HPLC and qualified by LC/MS interfaced to two soft ionization techniques--an atmospheric pressure ionization and an electron spray ionization (API-ES). Results demonstrated that C. acetobutylicum is capable of transforming RDX with H2 as the electron donor. The transformation followed a zero-order kinetics and the rates increased with increasing H2. RDX was transformed into several polar intermediates that could not be separated by reverse-phase HPLC and its molecular ions were unstable under the condition of commonly used electron impact detector. Using a polar and water immiscible solvent (ethyl acetate) and the softer MS ionization techniques, mass spectroscopy detected the presence of several RDX derivatives including mononitroso-, monohydroxylamino-, mononitrosomonohydroxylamino-, monoamino-, diamino-, and triamino-compounds. The presence of hydroxylamino compounds is analogous to the transformation of TNT and DNTs we elucidated previously.