The herbicide bentazone is recalcitrant in aquifers and is therefore frequently detected in wells used for drinking water production. However, bentazone degradation has been observed in filter sand from a rapid sand filter at a waterworks with methane-rich groundwater. The association between methane oxidation and removal of bentazone was investigated with a methanotrophic enrichment culture derived from methane-fed column reactors inoculated with that filter sand. Several independent lines of evidence obtained from microcosm experiments with the methanotrophic enrichment culture, tap water and bentazone at concentrations below 2 mg/L showed methanotrophic co-metabolic bentazone transformation: The culture removed 53% of the bentazone in 21 days in presence of 5 mg/L of methane, while only 31% was removed in absence of methane. Addition of acetylene inhibited methane oxidation and stopped bentazone removal. The presence of bentazone partly inhibited methane oxidation since the methane consumption rate was significantly lower at high (1 mg/L) than at low (1 μg/L) bentazone concentrations. The transformation yield of methane relative to bentazone normalized by their concentration ratio ranged from 58 to 158, well within the range for methanotrophic co-metabolic degradation of trace contaminants calculated from the literature, with normalized substrate preferences varying from 3 to 400. High-resolution mass spectrometry revealed formation of the transformation products (TPs) 6-OH, 8-OH, isopropyl-OH and di-OH-bentazone, with higher abundances of all TPs in the presence of methane. Overall, we found a suite of evidence all showing that bentazone was co-metabolically transformed to hydroxy-bentazone by a methanotrophic culture enriched from a rapid sand filter at a waterworks.