Recent investigations on water microbiology have shown that most of the biomass present in the drinking water network is located at the pipe walls as biofilms. Biofilms are organized in highly efficient and stable ecosystems and can be viewed as a survival mechanism; this way of life can provide protection from chemical, biological or physical stresses. Moreover, biofilms play a major role in the accumulation, protection and dissemination of pathogens through water distribution systems. Although filamentous fungi are especially adapted for growth on surfaces, fungal water biofilms have received less attention when compared with bacterial biofilms, thus remaining a lack of information in this field. The use of microscopy techniques associated with image analyses has become a valuable tool for in situ studies. Fluorescent in situ hybridization (FISH) and fluorescent dyes are non-invasive and non-destructive techniques which provide information on cell morphology, metabolism and phylogeny. In this work, we aimed to detect filamentous fungal biofilm in a water distribution system using FISH (EUK516 and FUN1429 probes) and Calcofluor staining in replaced pipes. We also presented a sampler developed to study in situ fungal biofilms formation in water distribution system. Calcofluor staining was a rapid and easy method to detect filamentous biofilms on pipes surfaces. Additionally, FISH provided phylogenetic information by the detection of eukaryotic and fungal cells. Ours results contributed to demonstrate the presence of fungi in water biofilms and emphasised that fungi play an important role in water biofilms and microbial water quality.