Comparing the fate and occurrence of aceclofenac, diclofenac and their human metabolites: Conventional treatment vs Membrane bioreactor treatment

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Aceclofenac ([2-(2',6'-dichlorophenylamino)phenyl]acetoxyacetic acid) is a member of the family of non-steroidal anti-inflammatory drugs (NSAIDs). This compound was designed to improve drug oral bioavailability and tolerance in humans. NSAIDs are extensively used as non-prescription drugs. Metabolism of aceclofenac in humans yields 4'-hydroxylated metabolites, and to a minor extent the conversion of aceclofenac to diclofenac takes place.

The non-steroidal anti-inflammatory drug diclofenac (DCF: 2-(2-(2,6-dichlorophenylamino)phenyl)acetic acid) constitutes one of the environmentally relevant pharmaceuticals having received considerable attention by the scientific community in the past couple of years due to frequent detection in monitoring surveys on sewage-impacted surface waters, associated with high consumption rates and low removal efficiencies during conventional activated sludge treatment in wastewater treatment plants (WWTP). Despite the great interest in the fate of DCF in engineered and environmental systems, two crucial components in the study on the overall fate of this polar chlorinated compound ($logD_{7.4}$: 1.11; $solubility_{Na\text{-}DCF}$: 1113 mg/L) have as of today been considered only marginally: the occurrence of human metabolites of DCF in environmental samples has not been documented at all.

The most widely used process for wastewater treatment is through conventional activated sludge (CAS) which utilizes the flocculent suspension of microbial mass. In this process, wastewater is mixed with the bacterial population in an aeration tank, which is then transported to a sedimentation tank where the flocculated biomass settles while the effluent goes on to the next step. Membrane bioreactor (MBR) is a system that combines the biological treatment of microorganisms and the membrane separation process, which replaces the secondary clarifiers, into a single step. The influent or feed water is mixed with the biomass, and this mixture is filtered through the membrane separating biomass from the treated water. There are several advantages of using MBR. The main benefit of MBR over CAS is that the amount of suspended solids remaining in the effluent of MBR is much lower that in CAS, resulting in a better quality treated water. The low turbidity of the effluent water makes it more amenable to further treatment. Another benefit of MBR results from its inherently high sludge age which allows for slow growing bacteria to develop, and lead to enhanced degradation of some compounds.

In full-scale WWTP relying on continuous activated sludge (CAS) treatment for the degradation of organic compounds, the biotransformation of the acidic DCF has

generally been estimated to be low based on comparisons of influent and effluent concentrations. Despite the general consensus viewing DCF as fairly recalcitrant to microbial attack, for an unbiased interpretation of differences in influent and effluent levels of the WWTP, it needs to be taken into consideration that conjugated DCF can be liberated during the biological wastewater treatment process. As mentioned above, a fraction of DCF is excreted from the human body as acylglucuronide which is potentially cleavable by β-glucuronidase enzymes, thus releasing the parent drug in its native form. In addition to this, a second possible source for DCF formation in the activated sludge tank is the ester hydrolysis of aceclofenac (ACF: 2-(2-(2-(2,6-dichlorophenylamino)phenyl)acetoxy)acetic acid), which is a potent anti-inflammatory and analgesic drug with efficacy similar to DCF but with improved gastro-intestinal tolerance.

Given the lack of field data on 4'-OH-DCF and the fact that ACF and 4'-OH-ACF are potential precursors of DCF and its major hydroxylated metabolite, respectively, the present study aimed at investigating still uncovered aspects in the environmental life cycle of DCF. Taken together, this work constitutes the first report on the occurrence of ACF and the human metabolites 4'-OH-DCF, 4'-OH-ACF in wastewater, underpinning the need of incorporating metabolites excreted by humans in monitoring surveys as part of a risk evaluation for environmentally relevant pharmaceuticals.