THE IMPROVEMENT OF THE BIODEGRADABILITY OF DICLOFENAC AND SULFAMETHOXAZOLE BY COMBINED COMETABOLISM AND GAMMA IRRADIATION TREATMENT

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Abstract

The two forms of most common biological purification used (activated sludge and biofilm) cannot effectively remove pharmaceuticals, such as diclofenac and sulfamethoxazole. However, a biological phenomenon called cometabolism can improve the removal efficiency. In the case of cometabolism, an easily degradable substrate (growth substrate) is added to the wastewater to be treated, which supplies the microorganisms with sufficient energy for biodegradation. Such easily degradable molecules (e.g. methanol, acetic acid, ethylene glycol) are also formed in the anaerobic steps of wastewater treatment systems and are utilized as growth substrates. The rate of oxidative degradation of pharmaceuticals (measured by using oxygen uptake rate) was shown to greatly increase in the presence of easily degradable growth substrates in the case of activated sludge. In the biofilm system which is another form of biological wastewater treatment, the rate of cometabolism remained low, most possibly because it has much narrower bacterial diversity than activated sludge. However, the efficiency of oxidative degradation can be significantly improved by using an advanced oxidation process, ionizing radiation treatment, before cometabolism. This combined treatment, irradiation and cometabolism is recommended for the degradation of recalcitrant organic compounds.

Introduction

The conventional wastewater treatment technologies are not suitable for removing hardly biodegradable micro-pollutants (xenobiotics), such as pharmaceuticals. These compounds often pass through the treatment systems in unchanged form and enter the surface waters [1, 2]. The pharmaceuticals, especially antibiotics remaining in the treated wastewater have several adverse effects on the environment: short-term and long-term toxicity, endocrine disruption or induction of resistant pathogens [3, 4].

The decomposition of xenobiotics can be enhanced by the stimulation of natural processes, so called cometabolism (co-oxidation), whereby a complex non-biodegradable molecule can be degraded in the presence of an easy-to-degrade substrate (growth substrate) [5]. Simple organic compounds (methanol, ethanol, formic acid, acetic acid, propionic acid, butyric acid, valeric acid, caproic acid or hexanoic acid, lactic acid, ethylene glycol, etc.) are formed by hydrolysis and microbiological fermentation from complex organic molecules in wastewater. Industrial by-products (acids and alcohols) can also be used for feeding the bacterial community. However, depending on the composition and diversity of the microbiota, other elements can be successfully utilized and it also affects the efficiency of cometabolism. The biomass of each wastewater treatment plant has a special composition, so its removal efficiency and biotransformation capability for micro-pollutants are also different [6, 7].

Several technological innovations are aimed at removing micro-pollutants, among these advanced oxidation processes (AOP) based on hydroxyl radicals ($^{\bullet}OH$) are highly effective [8, 9, 10]. This process eventually results in the formation of simpler, biodegradable (available to microorganisms) organic compounds.
The aim of this work was to improve the removal efficiency of the biological treatment for hardly biodegradable pharmaceuticals, namely sulfamethoxazole (SMX, antibiotic) and diclofenac (DCF, analgesic, non-steroidal anti-inflammatory drug). For this purpose AOP and cometabolism, as well as their combination were tested. The phenomenon of cometabolism was studied by the addition of simple growth substrates (methanol, acetic acid, ethylene glycol), and γ radiation was used as an advanced oxidation process at three different doses. The change in the bioavailability of the compounds was followed by respiratory tests on wastewater activated sludge and biofilm cultures.

Experimental

As an advanced oxidation method, γ irradiation was used with 0.5, 1.0 and 2.0 kGy doses. Irradiations were performed by a ^{60}Co (1.85PBq) SSL-01 panoramic type gamma source, with 9.4 kGy h^{-1} dose rate. The samples containing pharmaceuticals in 0.1 mmol L^{-1} concentration were continuously aerated and kept at room temperature during the treatment. H_2O_2 generated during ionizing radiation treatment was eliminated with dosing 0.25 g L^{-1} manganese(IV) oxide at pH 10.0 (stirred at 20 °C for 10 minutes). After filtration with 0.22 μm regenerated cellulose membrane filter the solutions were neutralized. This step is necessary to avoid toxicity and measurement interfering effects by elevated oxygen levels.
Organic matter content was expressed as chemical oxygen demand (COD) as the organic matter is oxidized by sulfuric acid and a known excess of potassium dichromate ($K_2Cr_2O_7$) at high temperature (2 h, 170°C). The remaining unreduced $K_2Cr_2O_7$ is titrated with ferrous ammonium sulfate (ISO 6060:1989).
The respiratory tests (on the basis of ISO 8192:1986 standard) were carried out with two types of wastewater bacterial cultures: activated sludge and biofilm biomass collected from the South-Pest Wastewater Treatment Plant. The cultures were used for testing freshly without storage and were washed several times with tap water. Oxygen consumption of the culture was measured in 300 mL Karlsruher bottles with an FDO® 925 oxygen sensor at 20 °C. The mixtures were made with tap water. The respiration intensity is expressed in oxygen uptake rate (OUR, mg O_2 L^{-1} h^{-1}).
The cometabolic effect is achieved by the addition of simple organic substrates with the same load levels in each bottle: 25 mg dry matter of biomass, 150 mg O_2 L^{-1} COD-equivalent substrate and 150 mL of irradiated pharmaceutical solutions. The blank test mixtures contained only biomass and substrates, while the control mixtures treated/untreated pharmaceutical samples and substrates without biomass. All data used to evaluate the results are corrected for endogenous OUR values (absence of pharmaceutical and growth substrate) and control values (currently tested pharmaceutical and growth substrate without biomass). The dissolved oxygen uptake is increasing in the presence of biodegradable compounds, and it is reduced in the presence of toxic substances.

Results and discussion

In order to study the combined effect of gamma radiation and cometabolism, we had to perform a number of preliminary experiments. First we had to identify the growth substrates that the bacterial cultures could utilize for the cometabolic biodegradation of the tested pharmaceuticals (DCF, SMX). If a bioculture (activated sludge, biofilm) is capable of efficient cometabolic degradation alone, the effect of gamma radiation is less detectable. According to this guiding principle we selected a poorly, a medium and a highly active

growth substrate and the less effective bacterial culture for further testing that worked similarly for both pharmaceuticals: methanol, ethylene glycol and acetic acid.
During the preliminary tests we found that activated sludge is efficient in cometabolism alone, the effect of irradiation treatment is further tested on the biofilm, which was less effective in cometabolism. The OUR data for the selected three substances are summarized in Fig.1.

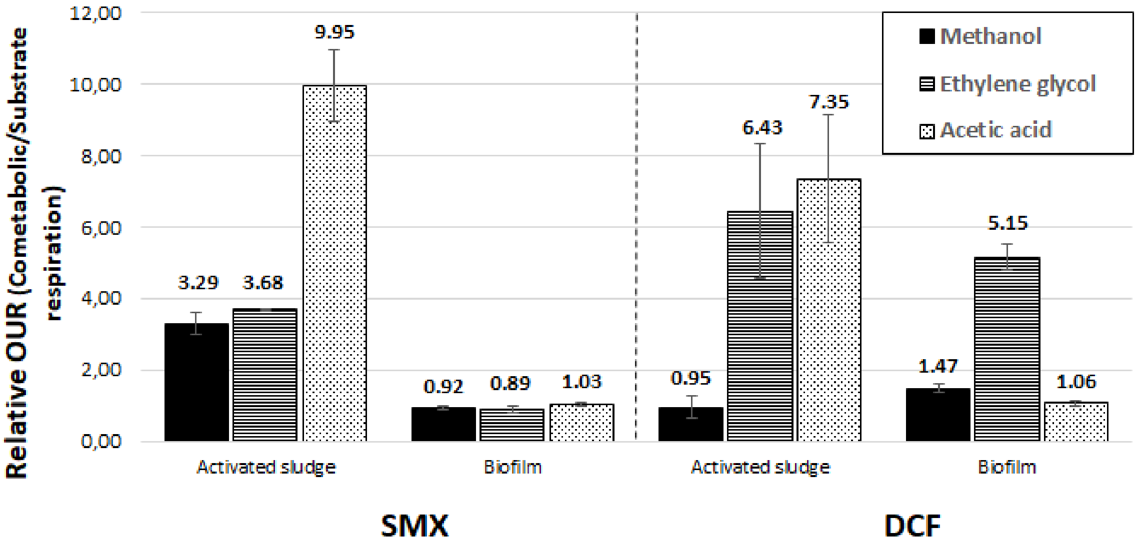

Figure 1. Relative OUR values for SMX (a) and DCF (b) with the three selected substrates using activated sludge or biofilm.

As Fig. 2 shows the untreated pharmaceuticals were not eliminated by the biofilm bacterial culture (0 mg L^{-1} h^{-1}, i.e. no metabolic activity). However, in the irradiated samples bioavailability of both pharmaceuticals was observed and the effect was enhanced with the dose. At 0.5, 1.0 and 2.0 kGy absorbed doses for SMX solutions 0.16, 0.27 and 0.35 mg L^{-1} h^{-1}, while for DCF 0.01, 0.14 and 0.31 mg L^{-1} h^{-1} oxygen consumptions were measured, respectively. The chemical oxygen demand (COD) decreased due to the irradiation treatment from 54 mg L^{-1} (not irradiated), to 52 mg L^{-1} (0.5 kGy), 37 mg L^{-1} (1.0 kGy) and 30 mg L^{-1} (2.0 kGy), for SMX, and from 48 mg L^{-1} (not irradiated) to 40 mg L^{-1} (0.5 kGy), 39 mg L^{-1} (1.0 kGy) and 34 mg L^{-1} (2.0 kGy) in the case of DCF (Fig. 2 inset). This indicates radiation induced degradation of the pharmaceuticals.
The post-irradiation OUR value with growth substrate are shown in Fig. 3. The values measured for the growth substrate (without irradiation) were subtracted. The OUR increment values of the figure show the effect of cometabolism and irradiation together. The higher the OUR values, the more pronounced the effect of irradiation on cometabolism.

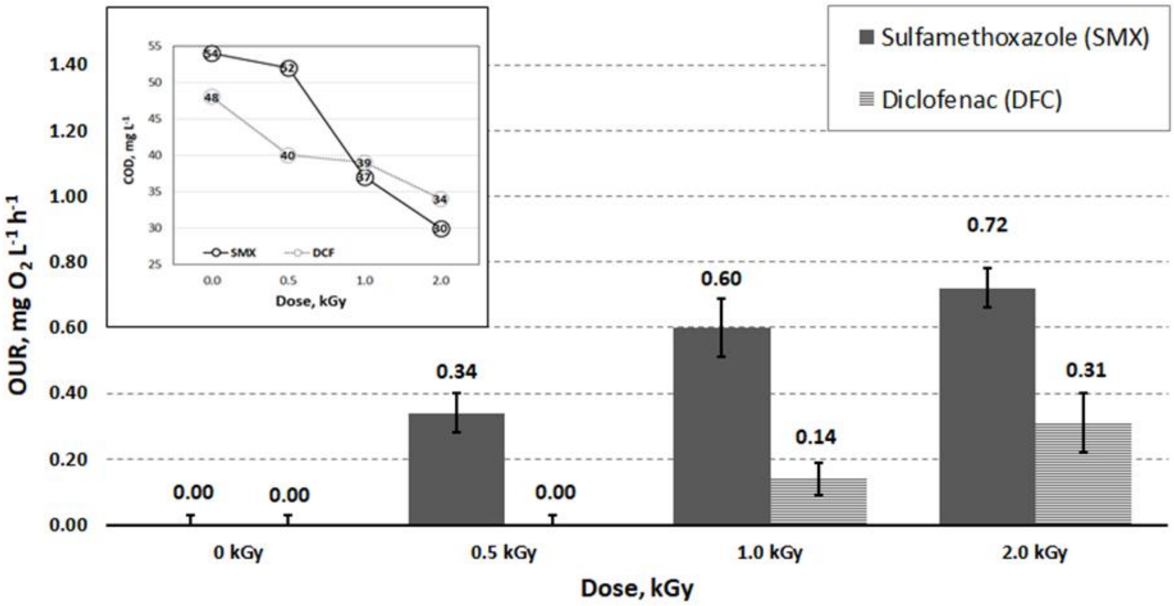

Figure 2. The effect of irradiation on oxygen uptake rate and chemical oxygen demand values (inset) of two pharmaceutical compounds (SMX and DFC).

The efficiency of the cometabolism can be increased by irradiating the samples with a relatively low dose. At 0.5 kGy absorbed dose, the increase in co-oxidative respiration for SMX was 0.47 mg L^{-1} h^{-1} with acetic acid, 1.60 mg L^{-1} h^{-1} with ethylene glycol and 2.24 mg L^{-1} h^{-1} with methanol. For DCF the increase was 0.53 mg L^{-1} h^{-1} with acetic acid, 2.05 mg L^{-1} h^{-1} with ethylene glycol and 4.19 mg L^{-1} h^{-1} with methanol. No improvement was observed at higher doses (Fig. 3). Above 0.5 kGy there is a very high oxidation percentage and there is considerable depletion in the organic material (Fig. 2, inset).

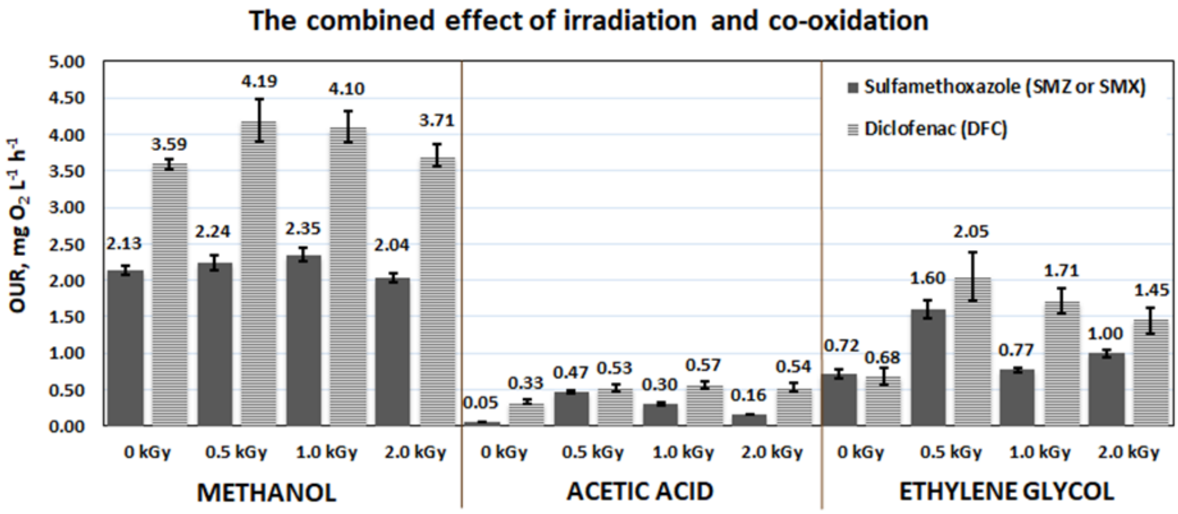

Figure 3. The effect of irradiation on oxygen uptake rate (OUR) increment in the presence of pharmaceuticals (SMX and DCF) and growth substrate (methanol, acetic acid and ethylene glycol) with the values measured for the growth substrate subtracted.

If acetic acid was added to the irradiated SMX solution, the OUR was almost ten times higher than without treatment (0.05 to 0.47 mg L^{-1} h^{-1}). Ethylene glycol mixed with irradiated DCF solution resulted in a triple respiration intensity relative to the untreated cometabolic effect (0.68 to 2.05 mg L^{-1} h^{-1}). The effect of treatment on the methanol as growth substrate is barely detectable (SMX: from 2.13 to 2.24 mg L^{-1} h^{-1}, DCF: from 3.59 to 4.19 mg L^{-1} h^{-1}). Based on these results, significant improvement can be achieved in the case of the inherently weak substrate. In the case of a highly active substrate, methanol, irradiation does not improve significantly the efficiency.

Conclusion

The biodegradation of sulfamethoxazole and diclofenac was stimulated separately by the two tested methods: cometabolism and advanced oxidation treatment (by high-energy ionizing radiation). Two type of bioculture (activated sludge and biofilm) and 10 organic molecules were tested, and finally methanol, acetic acid and ethylene glycol were selected as growth substrates for cometabolism by biofilm biomass. Both pharmaceuticals showed cometabolic effect, but it was really significant for activated sludge. The biodegradability of sulfamethoxazole and diclofenac in 0.1 mmol L^{-1} aqueous solutions was enhanced by irradiation. A further increase was observed when the two methods, cometabolism and irradiation were combined. The the efficiency of the cometabolism can be enhanced by irradiation up to 0.5 kGy. No improvement was observed at higher doses most possibly due to the depletion of organic molecules by irradiation. Acetic acid added to the sulfamethoxazole almost ten times, the ethylene glycol mixed with diclofenac three times increased the respiration intensity as compared to the values obtained without irradiation.

The use of advanced oxidation techniques combined with cometabolic technology can be a successful and cost effective development path for the removal of pharmaceuticals. These two methods can be combined at the end of the wastewater treatment technology as a fourth stage.

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