REMOVAL OF PHARMACEUTICALLY ACTIVE COMPOUNDS IN SEQUENCING BATCH REACTOR

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ABSTRACT

Biological treatment efficiency of six pharmaceutical compounds (acetazolamide, metronidazole, opipramole, piracetam, salicylamide and tinidazole) was evaluated using lab-scale sequencing batch reactor (SBR). Comparative biological degradation processes of two types of activated sludge from municipal and pharmaceutical industry sewage treatment plants were examined. Three different organic loadings (0.05 ÷ 0.2 g COD/g TSS·day) and reaction time on the efficiency of API (active pharmaceutical ingredient) decomposition were examined. Chemical oxygen demand, non-purgeable organic carbon as well as ammonium nitrogen contents were monitored by standard methods. Percentage of API decomposition was analysed by HPLC. The overall API removal efficiency was strictly dependent on the type of activated sludge origin. The main biodegradation products were identified using HPLC-MS, ^{1}H NMR and ^{13}C NMR methods as e.g. ({4-[3-(5*H*-dibenzo[*b,f*]azepin-5-yl]piperazin-1-yl}methyl)amine and (2-amino-1,3,4-thiadiazol-5-sulfonamide) for opipramole and acetazolamide, respectively.

Keywords: acetazolamide, opipramole, metronidazole, salicylamide, tinidazole, piracetam, sequencing batch reactor, biodegradation,

1. Introduction

In recent years, considerable attention was paid to the presence of organic micropollutants such as active pharmaceutical ingredients (API) in the aquatic environment due to their negative impact on public health and aquatic ecosystems (Parrott and Blunt, 2005; Mehinto *et al*., 2010). Numerous papers have pointed out API presence as common constituents of effluents from wastewater treatment plants (WWTPs) (Kosma and Lambropoulou, 2010, Verlicchi *et al*., 2012; Padhye *et al*., 2014). This is the result of various efficiencies of API removal by conventional activated sludge process. For example the carbamazepine and ibuprofen are eliminated in <10% and >90% in municipal WWTPs, respectively (Joss *et al*., 2005). The elimination of APIs in WWTPs can be attributed to their biodegradation as well as adsorption processes. All of them depend on APIs chemical and physical properties, namely solubility, volatility, adsorptivity, absorbability, biodegradability, polarity and stability (Le Minh *et al*., 2010; Ziylan and Ince, 2011). The biological degradation of pharmaceuticals occur by direct metabolism or co-metabolism, which is strictly dependent on the type of microorganisms living in the inoculum. There are many methods used to improve the wastewater treatment process, as e.g. advanced oxidation processes (AOP) (Klavarioti *et al*., 2009, Oller *et al*., 2011). However, they are frequently used as pre-treatment processes of wastewater for their biological processing. Hence, improving the activated sludge processes is so important. Great potential in the treatment of highly concentrated industrial and municipal wastewater lies in using anaerobic-aerobic sequencing batch reactor (SBR). A very important advantage is its construction consisting of one tank (without the need for clarifier), minimum size, low cost and flexible operation of aeration and control.

The aim of this study was to investigate the behaviour of six pharmaceutical compounds during their treatment in SBR equipped with activated sludge from municipal and industrial

(pharmaceutical company) wastewater treatment plant. The basic parameters characterizing the biological wastewater treatment process, namely COD, NPOC, $N\text{-}NH_4^+$ were examined. Finally, the structures of selected products of biotransformation were determined.

2. Materials and methods

Acetazolamide (ACT), opipramole dihydrochloride (OPI), metronidazole (MTR), salicylamide (SA), tinidazole (TND) and piracetam (PIR) were donated by local pharmaceutical companies. Sodium hydroxide, diethylene glycol were purchased from Sigma Aldrich. All solvent were HPLC grade purched from Fluka. Activated sludges for pharmaceuticals' degradation were obtained from the municipal (Gdańsk) and from local pharmaceutical company wastewater treatment plant.

The biological degradation tests were conducted out in a 5 L volume glass model reactor, provided with a stirrer, aeration system, pH and temperature sensors. The activated sludge before using was aerobically conditioned for 24 h, keeping oxygen level at 3 mg L^{-1}. Aqueous solution of one of the investigated pharmaceuticals (10 mM) was mixed with activated sludge (the organic loading: 0.05 ÷ 0.2 g COD/g TSS·day) and kept them in contact for 5 min while stirring. Then a sample of this mixture was taken, filtered with a microfilter 0.22 µm pore size and chemical oxygen demand (COD), non-purgeable organic carbon (NPOC), ammonia nitrogen ($N\text{-}NH_4^+$) as well as API concentration were measured. After this time, the system was aerated for 19 h. Then aeration system was shut down for 3 h. After full separation of the bio-sludge, the supernatant was examined as above. The sewage sludge samples were homogenized, frozen, lyophilized, extracted by appropriate mixture of organic solvents and analysed using HPLC. Diethylene glycol, a biodegradable substance, was used in a parallel run in order to check the functional ability of the activated sludge.

The total suspended solids (TSS) of the sludge samples were determined according to Standard Methods (APHA, 2005). The chemical oxygen demand and ammonia nitrogen were determined by HACH LANGE cuvette tests. The non-purgeable organic carbon was measured by a TOC-V CSH Shimadzu analyzer. The contents of residual parent drug concentration was determined by high performance liquid chromatography (HPLC). An HPLC 1200 series system (Agilent Technology, USA) was equipped with diode-array detector. Analyses were performed on Zorbax SB C18 (250 x 4.6 mm, 5 µm, Agilent Technologies) reverse-phase column operated at 25 °C, with flow rate 1 ml/min of mobile phase (eluent A: 0.05 M $HCOONH_4$ and eluent B: acetonitrile). The analytes were separated at the following gradient elution conditions (min/A%): 0/99, 30/70, 45/25, 46/99, 53/99. UV absorption was monitored at 254 nm for ACET, SA and OPI as well as at 310 nm for MTR and TND. For PIR, as a mobile phase was used 0.01% $HClO_4$ (V/V) as eluent A, acetonitrile as eluent B and methanol as a eluent C. PIR was separated with the following gradient elution conditions (min/A%/B%): 0/97/1, 2.7/96/2, 3/5/80, 4.7/5/88, 5.5/97/1, 12/97/1. UV absorption was monitored at 210 nm.

Isolation and identification of intermediate products were performed by UPLC-MS (4000 Q-TRAP used ionization method ESI (Electrospray Ionization), ^1H NMR and ^{13}C NMR spectra registered on Varian Instrument at 500 MHz and 125 MHZ, respectively. Each experiment was repeated 3 times. The results are presented as the avarage values.

3. Results and discussion

3.1. Biodegradation study

Laboratory biodegradation tests were performed in separate batches with acetazolamide, opipramole, metronidazole, salicylamide, tinidazole and piracetam (Figure 1.) to investigate the biodegradation of these pharmaceuticals by MAS (activated sludge from municipal wastewater treatment plant) and IAS (activated sludge from industrial wastewater treatment plant). The effect of organic loading (0.05 ÷ 0.2 g COD/g TSS·day) and reaction time on the efficiency of API removal was determined. This selection of pharmaceuticals was based on the documented high production of these compounds in a local pharmaceutical company.

The degradation profiles of API in the biological tests are shown in Figure 2. The removal percentage of pharmaceuticals varied from insignificant (0%, TND) to 100% (SA and PIR). Nonetheless, for the most of analysed cases, activated sludge of industrial origin is characterized by higher efficiency of pharmaceuticals elimination than municipal sludge. For example, ACT was decomposed in 99% by activated sludge, with the use of inoculum taken from SBR treating real wastewater that includes the tested pharmaceutical. This result was obtained after 8 h when the organic loading of 0.1 g COD/g TSS·day was applied. The municipal activated sludge provides only 9% of ACT transformation even after 24 h of the biological treatment.

The biological transformation of MTR on IAS increased progressively with the incubation time. For the organic loading 0.05 g COD/g TSS·day, biodegradation of analysed pharmaceutical compound was 51%, 75% and 97% after 4 h, 8 h and 24 h, subsequently. Microorganisms presented in MAS appeared to be incapable for removing MTR. API removal of 51% was achieved at 0.05 g COD/g TSS·day. On increasing the organic loading rate to 0.2 g COD/g TSS·day, MTR removal rates were inhibited markedly. The reasonably poor performance of the SBR at higher organic load can be attributed to the presence of high concentration of relatively toxic and inhibitory substances in the wastewater. MTR probably inhibits the performance of ammonia oxidizing bacteria. It is noted due to increase ammonia values to 42% and 24% in a lab-scale SBR system for IAS and MAS, respectively. Moreover, COD removal was not affected (13% for IAS and 10% for MAS), suggesting that heterotrophic bacteria were robust to this compound.

Piracetam has been reported to be eliminated in 100% by both IAS and MAS during total 24 h cycle period with an organic loading rate 0.05 g COD/g TSS·day. Although, SBR methods were gratifying in COD removal (MAS: 75% and IAS 74%), the nitrogen ammonia significantly increased. This situation can be explained by fast progress in ammonification process, during insufficient work of ammonia-oxidizing batteries which are vital to the maintenance of proper functioning of wastewater treatment plants.

The industrial activated sludge occurred to be efficient during SA biodegradation. Results have shown that general SA decomposition ended after 4 h of the process even under the highest API loading. COD dropped sharply from 421 to 150 $mg \cdot L^{-1}$ in the first 8 h and reached 86 $mg \cdot L^{-1}$ after 24 h. Even thought, SA occurred to be well assimilable by inocullum' microorganisms, the completely mineralized was not observed (MAS: 79% and IAS 68%).

Elimination of micropollutants in SBR system can occur by biodegradation (biotransformation and mineralization) or by sorption on biological sludge. It has been reported, that from all examined compounds, OPI was particularly adsorbed onto biological sludge. Thus, the biological transformation (L_{Biol}) calculated from the difference in the loads of the influent (L_{in}), effluent (L_{out}) and excess sludge (L_{sld}), according to Eq. (1), after 24 h of the process reached 94%, 87% and 61% for IAS at the organic loading 0.05, 0.1 and 0.2 g COD/g TSS·day, respectively. MAS characterises in lower elimination capability of OPI with the results: 96%, 79% and 32% (gradually decreasing with the increasing of organic loading). For processes characterized by the highest removal efficiency, the reduction of NPOC and COD values was insignificant (for IAS: 10% (NPOC), 46% (COD) and for MAS: 2% (NPOC) and 25% (COD)).

$$L_{Biol}=L_{in}- L_{sld} – L_{out} \quad (1)$$

Among the analysed API, tinidazole was the most resistant to biodegradation and was not removed by both municipal and industrial origin activated sludge. NPOC was propped in only 2% and 1.50%, while ammonium nitrogen increased in 40 and 18 % for MAS and IAS, respectively. Tinidazole belonging to the bacteriostatic compounds may result in drop of tolerance to the sludge biocoenosis, causing its poisoning. The inhibition of biochemical processes, especially nitrification is observed. Moreover, TND can disturb the microorganisms' activity (e.g. respiratory and enzymatic processes), reduced the diversity in bacterial communities. Due to the low biodegradability of TND, it is frequently detected in aquatic environment (Diwan *et al*., 2009).

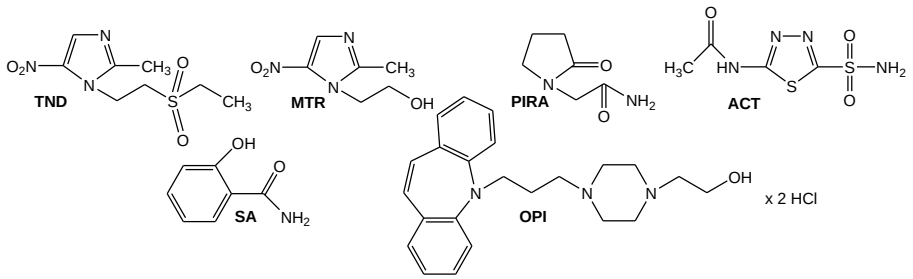

Figure 1: Chemical structures of tested pharmaceuticals.

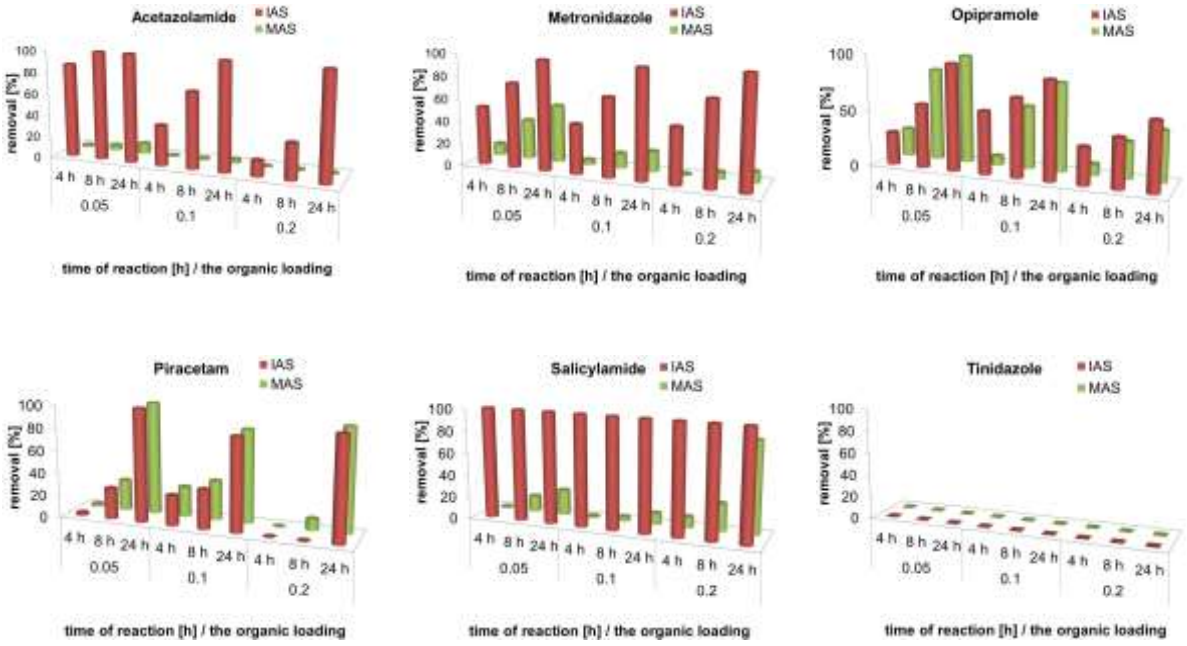

Figure 2: Comparison of percentage API decomposition by IAS and MAS (activated sludge from industrial and municipal wastewater treatment plant, respectively). Effect of the organic loading and process time on the efficiency of API removal.

3.2. Biotransformation products

Leading the biological SBR processes, it is also of interest to get information about the main biotransformation products in order to know what kind of compounds can be accumulate in the environment. After 24 h of biodegradation tests, ACT, OPI and MTR were transformed into new compounds revealing higher polarity than original APIs. Proposed chemical structures of their metabolites are presented in Figure 3. During ACT biological treatment its deacetylation reaction is observed. The formed product: 2-amino-1,3,4-thiadiazole-5-sulfonamide points out several biological activities and antibacterial properties (Akbas and Berber, 2006). Transformation of MTR via microorganism living in MAS as well as IAS is led to compound number 2. It was discovered in positive ion mode with its molecular weight of 185 with a correspondent $[M+H]^+$ ion of 186. This MTR's metabolite named as 1-metronidazole acetic acid (2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetic acid), has been already reported as one of the urinary oxidative metabolites found in the human urine (Stambaugh *et al*., 1968). The structure of OPI biotransformation was identified as the product of enzymatic reactions: oxidation, decarboxylation and amination and termed ({4-[3-(5H-dibenzo[*b,f*]azepin-5-yl]piperazin-1-yl}methyl)amine. This microbial metabolites of OPI has not been reported before.

It is interesting, that all obtained biotransformation products have an affinity to activated sludge. The amount of the adsorbed products for about 10% of fully formed products (for each system) was accounted.

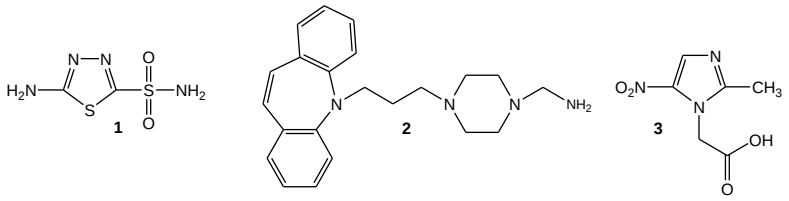

Figure 3: Chemical structures of biotransformation products.

4. Conclusions

This study elucidated the performance of SBR system in biodegradation processes of six commonly known active pharmaceutical ingredients. The efficiency removal of API by two types of activated sludge from municipal and pharmaceutical industry sewage treatment plants were examined. The results indicated, that TND has a significant persistency on biotransformation processes. An overview of API decomposition indicated, that biological degradation using MAS contributes only to a limited extent to reduction of ACT, MTR or SA, while IAS provided the complete elimination of examined APIs. It is concluded that, introduction of pharmaceuticals into a municipal sewage treatment plant, which are characterized in low biodegradability of many micropollutants might contribute a serious threat to the environment. Therefore, systematical control of municipal wastewater treatment plants in terms of API removal is required.

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