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Aerobic biodegradation of the sulfonamide antibiotic sulfamethoxazole by activated sludge applied as co-substrate and sole carbon and nitrogen source

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HIGHLIGHTS

- Elimination of sulfamethoxazole (SMX) turned out to be based on biodegradation.
- Activated sludge communities utilize SMX as carbon and/or nitrogen source for growth.
- SMX biodegradation is enhanced in the presence of a C source and N deficiency.
- With SMX as co-substrate 3-amino-5-methyl-isoxazole represents the main stable metabolite.
- SMX as sole C and N source maybe yields hydroxyl-N-(5-methyl-1,2-oxazole-3-yl)benzene-1-sulfonamide as further metabolite.

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ABSTRACT

Potential aerobic biodegradation mechanisms of the widely used polar, low-adsorptive sulfonamide antibiotic sulfamethoxazole (SMX) were investigated in activated sludge at bench scale. The study focused on (i) SMX co-metabolism with acetate and ammonium nitrate and (ii) SMX utilization when present as the sole carbon and nitrogen source. With SMX adsorption being negligible, elimination was primarily based on biodegradation. Activated sludge was able to utilize SMX both as a carbon and/or nitrogen source. SMX biodegradation was enhanced when a readily degradable energy supply (acetate) was provided which fostered metabolic activity. Moreover, it was raised under nitrogen deficiency conditions. The mass balance for dissolved organic carbon showed an incomplete SMX mineralization with two scenarios: (i) with SMX as a co-substrate, 3-amino-5-methyl-isoxazole represented the main stable metabolite and (ii) SMX as sole carbon and nitrogen source possibly yielded hydroxyl-N-(5-methyl-1,2-oxazole-3-yl)benzene-1-sulfonamide as a further metabolite.

1. Introduction

The discharge of antibiotics into the environment has become a major concern as this group of pharmaceuticals is not only prone to influencing microbial communities by its mode of action (Fent et al., 2006), but also because of the risk of a worldwide dispersal of concomitant resistance genes (Witte, 2004; Agersø and Petersen, 2007; Szczepanowski et al., 2009). Recent monitoring programs have revealed the presence of antibiotics in various environmental compartments such as water bodies, sediments, and soils (Heberer, 2002; Thiele-Bruhn and Beck, 2005; Ternes and Joss, 2007). Besides diffuse agricultural input (Jjemba, 2002) and landfill discharge (Heberer, 2002), antibiotics mainly enter the environment via wastewater (WW) and wastewater treatment plant (WWTP) effluents. Poorly adsorptive polar substances such as sulfonamides are readily found in water bodies such as surface waters (Hirsch et al., 1999), bank filtrates (Heberer et al., 2008), and groundwater (Sacher et al., 2001; Underwood et al., 2011). The concentration levels (see review, Kümmerer, 2009) range from 370 to 2000 ng L^{-1} in WWTP effluents, from 40 to 1900 ng L^{-1} in surface water and from 20 to 470 ng L^{-1} in groundwater/bank filtrate up to mg L^{-1} in highly contaminated groundwater (Holm et al., 1995).

Considering a 50% minimum inhibitory concentration (MIC_{50}) for e.g. sulfonamide antibiotic sulfamethoxazole (SMX), ranging from 0.002 to 256 mg L^{-1} depending on the bacteria species (Al-Ahmad et al., 1999), then the environmental concentrations found might influence microbial community activity.

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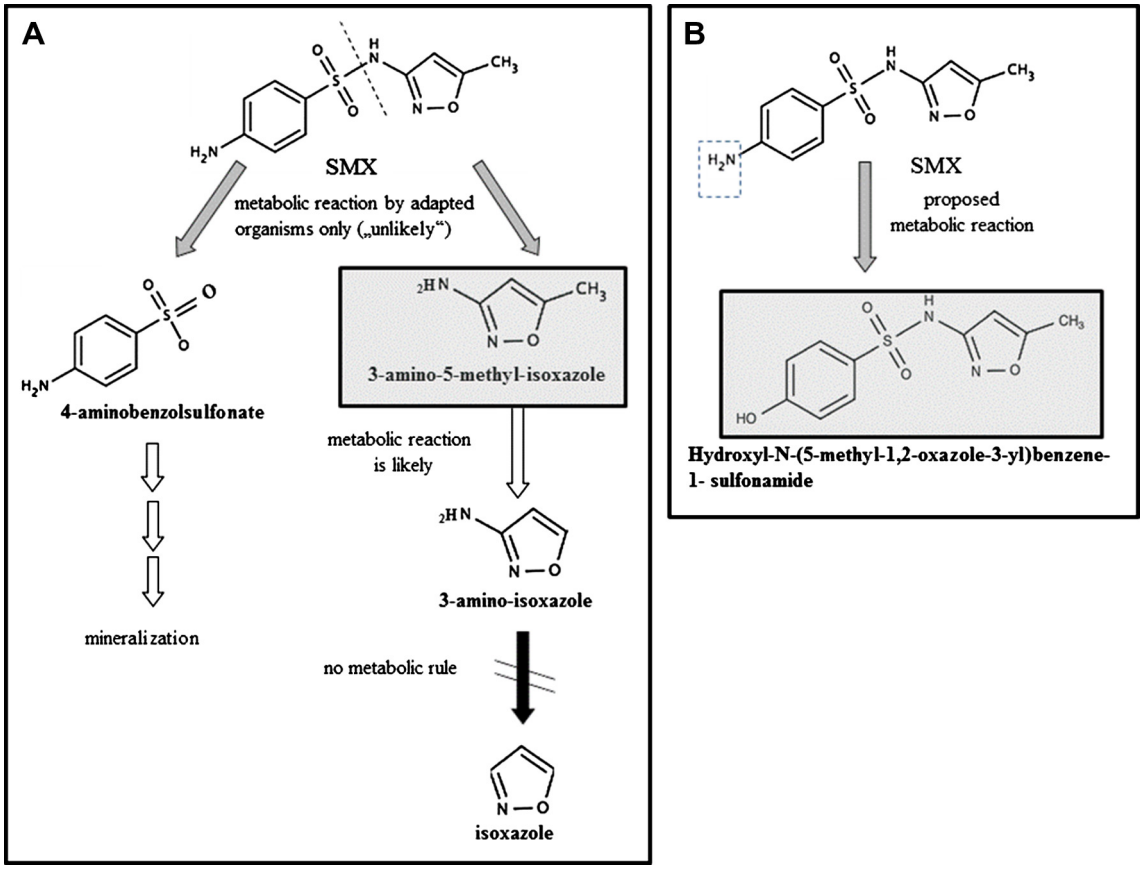

Fig. 1. Chemical structure of sulfamethoxazole (SMX) and its potential metabolites predicted by two postulated aerobic biodegradation pathways (A) according to biocatalysis/biodegradation database of the University of Minnesota (UM-BBD, http://umbbd.msi.umn.edu, (Gao et al., 2010)) and (B) according to Gauthier et al. (2010). Metabolites marked in grey were found or postulated in the present study.

SMX (Fig. 1) is a low adsorptive, polar sulfonamide antibiotic known as contaminant in drinking water resources. Hence its fate in aqueous environments is of high interest. SMX mainly enter wastewater via human excretion as unmodified SMX (13–5%) or as its human transformation products N-acetyl-SMX (43–55%) or N-SMX-glucuronide (10–13%) (van der Ven et al., 1995). Both human transformation products are readily cleaved back to the SMX molecule while passing through the sewer system or during WW treatment (Göbel et al., 2005). Previous investigations of activated sludge have shown elimination of SMX under different WW treatment conditions. Because of its low adsorptivity, it is postulated that SMX reduction is mainly due to microbial activity. However, proper balancing studies are rare (Golet et al., 2003; Carballa et al., 2007), presumably because of missing information regarding its metabolic fate and produced metabolites. In general, the literature pertaining to SMX elimination in WWTPs and natural aqueous environments is marked by inconsistent results. In WW treatment for example, low hydraulic residence times and sludge ages at high loadings were shown to be unfavorable for SMX elimination (Kreuzinger et al., 2004). On the other hand, Perez et al. (2005) describe a high-load sludge which readily eliminated sulfonamides. However, low-load nitrification conditions with a high sludge age were proven to be disadvantageous as a highly active community is necessary for good elimination rates (Göbel et al., 2007). Additionally, nutrient deficiencies (i.e. carbon and/or nitrogen) were pointed out as possibly being advantageous for microbial SMX reduction (Drillia et al., 2005). Studies on SMX elimination in natural systems, such as bank filtrates, also provided contradictory findings (Baumgarten et al., 2011). This is supposedly because elimination depends on various environmental factors such as in situ redox potential, available nutrients, soil characteristics, seasonal temperature, and light variations. Besides biological processes, literature indicates that abiotic SMX elimination (chemical oxidation (Sharma et al., 2006) or photochemical degradation (Andreozzi et al., 2003; Boreen et al., 2004; Trovó et al., 2009) is also possible. It has been shown that in natural environments different redox potentials due to the presence of nitrate, sulfate, humic acids, or chinone soil fractions can improve xenobiotic biodegradation (Field et al., 2000; Cervantes et al., 2008).

Unfortunately, actual literature on SMX reduction (Kreuzinger et al., 2004; Göbel et al., 2005, 2007; Perez et al., 2005) mostly refers to 'elimination' rather than effective 'biodegradation' with defined metabolites. Earlier and recent lab scale test using activated sludge as inoculum showed a high variation in aerobic biodegradation removal capacity after 48 h from <20% (Joss et al., 2006) up to 39% (Li and Zhang, 2010). In batch soil tests detected SMX half-life ranged from 1 to 11 d (Baumgarten et al., 2011; Lin and Gan, 2011). Only rarely is the literature substantiated by biological action of defined microorganisms (Gauthier et al., 2010; Larcher and Yargeau, 2011; Bouju et al., 2012). Knowledge gaps with respect to produced metabolites hinder proper balancing studies. However, appropriate experiments, which are based on realistic test conditions such as continuous food supply and sufficient adaption periods, should facilitate assessment of SMX biotransformation in the natural environment.

The aim of the present work was to elucidate the fate of the low-adsorptive polar model antibiotic sulfamethoxazole (SMX), during aerobic WW treatment, i.e. the fate of SMX with oxygen as the terminal electron acceptor. This redox condition is described