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Biodegradation of the antiviral tenofovir disoproxyl by a cyanobacteria/bacteria culture.

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Abstract

Tenofovir disoproxil fumarate (TDF) is an antiretroviral drug extensively used by people living with HIV/AIDS. TDF molecule is hydrolysed in vivo and liberate the tenofovir, the active part of the molecule. Tenofovir is a very stable drug and the discharge of its residues into the environment can potentially lead to risk for aquatic species. This study evaluated the TDF biodegradation and removal by cultures of Microcystis novacekii, non axenic, it presented the bacteria Pseudomonas pseudoalcaligenes associated. Concentrations of TDF 12.5, 25.0 and 50.0 mg.L-1 were used to the tests. The process occurred in two stages. In the first 72 hours, TDF was de-esterified, forming the tenofovir monoester intermediate by abiotic and enzymatic process associated in extra cell medium. In a second step, the monoester was removed from the culture medium by intracellular processes. At the end of the experiment 88.7 to 94.1% of TDF and its monoester derivative were removed from the culture medium over 16 days. This process showed higher efficiency to the TDF removal at the concentration 25 mg.L-1. The tenofovir or others by-products of TDF were not observed in the test conditions. Tenofovir isoproxil monoester has partial antiviral activity and has shown to be persistent, maintaining a residual concentration after 16 days, indicating the need to continue the research on methods of this product total removal from the aquatic environment.

Introduction

In recent decades problems related to water contamination have become a global concern. In this context, the dispersion of pharmaceutical residues in the aquatic environment has been the target of numerous studies (Ortúzar et al. 2022; Mojiri et al. 2022; Wilkinson et al. 2022). Drugs are biologically active molecules and their unpredictable effects on species exposed in the aquatic environmental may represent a potencial risk to the aquatic ecosystem (Isidori et al. 2007; Fick et al. 2010; Fabbri 2015; Agunbiade and Moodley 2016; Rzymski et al. 2017).

Studies have indicated the presence of drug residues in several environmental matrices, such as soil, sewage and surface and treated waters (Kümmerer 2001; Fent et al. 2006; Hernando et al. 2006; Yamamoto et al. 2007; González et al. 2012; Wood et al. 2015; Hanna et al. 2018; Mahmood et al. 2019;). Among the drugs, the antiretroviral (ARVs) are of particular concern, since some of them act in viral DNA synthesis and, potentially, may have effects on the replication process of others organisms (Poirier et al. 2004; Olivero, 2007; Brambilla et al. 2012).

In according to United Nations on HIV/AIDS (UNAIDS, 2021), currently 37,7 million people worldwide are living with HIV/AIDS and the control of the pandemic depends on continuous antiretroviral therapy. Thus, due to widespread use, the presence of ARVs in the environment have been reported in different countries (Prasse et al. 2010; K'oreje et al. 2012; K'oreje et al. 2016; Bottoni and Caroli 2018; Fekadu et al. 2019).

Tenofovir disoproxil fumarate (TDF) is an ARV of great importance because it is one of the first-line drugs, combined with other antiretroviral, in HIV/AIDS treatment. Tenofovir is an analogue of the nucleotide adenosine 5'-monophosphate and acts by inhibiting the reverse transcriptase enzyme, necessary for viral replication in human cells. Due to the low lipid solubility (Kearney et al. 2004) of tenofovir, it is administered

as a prodrug, in esterified form, and it is de-esterified intracellularly to release the active portion of the molecule, tenofovir (Fung et al. 2002; Leite et al. 2015) (Fig. 1).

Figure 1 Metabolism of the prodrug tenofovir disoproxil with formation of the de-esterification products: tenofovir monoester and tenofovir

About 80% of the dose of TDF administered to humans is eliminated in the active form, as a deesterified product, tenofovir (Fig. 1), without undergoing further metabolism (Kearney et al. 2004). The tenofovir molecule is very stable (Agrahari et al. 2015), which makes the treatment for its removal from the aquatic environment a challenge, both in the case of industrial effluents and domestic sewage.

Effluent treatment technologies that employ microorganisms are the most used processes for the degradation of organic compounds (Chan et al. 2022). In recent years, the use of cyanobacteria and microalgae in waste treatment has aroused interest (Gonçalves et al. 2017; Chan et al. 2022; Touliabah et al. 2022). These are photosynthetic organisms and the presence of these groups in consortia makes the process more sustainable compared to technologies that use bacteria intercropping.

Cyanobacteria species have also the ability to degrade different types of chemical compounds by mixotrophyc metabolisms acting together with bacteria in the degradation of chemicals in environment. (Wu et al. 2012; Chekroun et al. 2014; Abinandan and Shanthakumar, 2015; Tolboom et al. 2019).

Cyanobacteria of the genus Microcystis have presented interesting results in biodegradation studies of several substrates. These species show resistance and rapid responses to eutrophication (El-Bestawy et al. 2007; Xiao et al. 2018), possibly by acclimatation or adaptation to the adverse conditions (Tandeau de Marsac & Houmard, 1993; Zeng et al. 2009).

Microcystis is one of the most commonly found genera in Brazil (Bicudo and Menezes, 2006) which justifies evaluating the potential of Microcystis species, local for biodegradation processes. Cultures of *Microcystis novacekii* has been studied and has demonstrated its ability to remove different types of pollutants (Fioravante et al. 2012; Campos et al. 2013).

Cyanobacteria usually present themselves in the environment associated with bacteria through interactions considered mutualistic, where cyanobacteria provide heterotrophic bacteria with organic matter to sustain their growth and oxygen for greater efficiency of aerobic metabolism. At the same time, the bacteria provide the cyanobacteria with carbon dioxide and essential nutrients for their metabolism. Thus, these associations are fundamental for the cycling of carbon and other essential ions to the aquatic environment (Cole, Likens and Strayer 1982; Lutzu and Dunford 2018; Zheng et al. 2018; Ye et al. 2020). The mutualistic interactions between these organisms make it very difficult to maintain axenic cyanobacterial strains in biodegradation studies (Zheng et al. 2018; Silva et al. 2019).

There are no studies on the removal of TDF from the aquatic environment by cyanobacteria culture, justifying the study of the potential of a *M. novacekii* culture to remove this antiviral. Thus, the aim of this

study was to evaluate the process of biodegradation of tenofovir disoproxil using a non-axenic culture of *M. novacekii* isolated from a Brazilian lake.

Materials And Methods

Reagents and Chemicals

The tenofovir disoproxil fumarate used in the experiments was obtained from Nortec Química (lot 507034) as a white, amorphous solid. The drug was analysed and certified by the Department of Quality Control of the Ezequiel Dias Foundation (FUNED). The reagents, solvents and other chemicals used were of analytical or high-performance liquid chromatography (HPLC) grade. All the solution were prepared using type 1 water.

M. novacekii culture

A cyanobacterium strain, *Microcystis novacekii* (Komárek) Compère, was isolated from water samples collected in Dom Helvécio Lake, in the Rio Doce State Park (42º, 35', 595"; 19º, 46', 419"), Minas Gerais, Southeastern Brazil) in May 2004. For the isolation of *Microcystis novacekii* strains pipetting and dilution series of lake water samples were used (Lourenço, 2006). The species was isolated in pure form and cultivated in WC (Wright's cryptophytes) culture medium (Guillard and Lorenzen, 1972; Andersen et al. 2005). The procedure rendered a non-axenic unialgal culture which has been kept in the culture with a WC medium at pH 7 and a controlled temperature of 25 ± 2°C under a fluorescent light of 110.5 μmol photons m^{-2} s^{-1} and a 12h photoperiod. The non-axenic *M. novacekii* strain has been kept in culture in the algae and cyanobacteria bank of the Laboratory of Limnology, Ecotoxicology and Aquatic Ecology at the Institute of Biological Sciences of the Federal University of Minas Gerais (LIMNEA-ICB-UFMG). *M. novacekii* strain was tested for the presence of microcystin to verify the toxigenic potential, using enzyme linked to immunosorbent assays and the polymerase chain reaction to amplify the mcyB gene in the DNA of this strain. Both results were negative for microcystin.

During the experiments, we observed the presence of *Pseudomonas pseudoalcaligenes* in culture. The bacterium associated with *M. novacekii* was identified by Neoprospecta Microbiome Technologies Company using next-generation amplicon sequencing (NGS) and Neobiome software.

M. novacekii culture medium

ASM1 medium (Gorham et al. 1964) buffered by the addition of 750 $mg.L^{-1}$ 3-(N-morpholino) propanesulfonic acid (MOPS), pKa 7.2, was used for *M. novacekii* culture. The pH was adjusted to 7.0 with 0.1 $mol.L^{-1}$ HCl or NaOH solution. The composition of the ASM1 medium was ($mg.L^{-1}$): $NaNO_3$ (170.00), $CaCl_2$ (29.00), $MgCl_2.6H_2O$ (41.00), $MgSO_4$ (49.00), K_2HPO_4 (8.70), $Na_2HPO_4.12H_2O$ (17.80), $CoCl_2.6H_2O$ ($9.5x10^{-3}$), $CuCl_2.2H_2O$ ($6.5x10^{-4}$), $MnCl_2.4H_2O$ (0.69), $ZnSO_4.7H_2O$ (0.35), H_3BO_3 (1.24), $FeCl_3.3H_2O$ (0.54), EDTA Na_2 (3.72).

TDF biodegradation by M. novacekii

The pre culture of *M. novacekii* was prepared by inoculation of *M. novacekii* to a flask containg ASM1 medium. This flask was incubated under controlled temperature (25°C). Cell density was evaluated by optical density (680 nm) (Ma et al. 2005) and was monitored until culture reached approximately 10^6 cell.mL^{-1}. The TDF biodegradation tests in culture of the cyanobacterium *M. novacekii* were carried out following the OECD protocol - Guidelines for testing of chemicals (2003) with adaptations. The TDF solution (2.5g.mL^{-1}) was added to test flasks containing 100 mL of *M. novacekii* culture with a cell density approximately of 10^6 cell.mL^{-1} to obtain final concentrations of 50.00, 25.00, 12.50 mg.L^{-1}. Cyanobacteria culture was used as growth control. To determine the stability of TDF in ASM1 medium, an uninoculated sterile medium in the same concentrations of the test flasks was prepared. The test flasks and controls were transferred to a shaking table and were incubeded to 16 days, under controlled temperature (25 ± 2°C) and 5000 lx (c. 11 W/m^2) illumination from cool-white fluorescent lamps. All experiments were conducted in triplicate.

Extraction of TDF and its metabolites from culture medium

After 1, 3, 7 and 16 days, 5.00 mL of the sample were removed from test flasks, filtered (0,45 µm -Millipore) and the aqueous portion was submitted to solid-phase extraction.The samples were subjected to solid-phase extraction using a Phenomenex Strata-X® cartridge (Phenomenex, USA). The cartridges were conditioned with 5 mL of type 1 water. The samples were transferred to the cartridge and eluted with 5 mL of 5% methanol solution, followed by 5 mL by pure methanol. The eluate was filtered (Millipore Filter, 0.22 µm pore size), transferred to a vial with an insert and injected into a liquid chromatographer coupled to a mass spectrophotometer for HPLC quadrupole time-of-flight mass spectrometry (HPLC/Q-TOF-MS) analysis.

HPLC/Q-TOF-MS HPLC analysis

A HPLC–QTOF-MS system (6540 UHD Accurate Mass Q-TOF LC/MS equipped with Agilent Mass Hunter Workstation Data Aquisition software) was used and experimental conditions were Zorbax Eclipse Plus C18 column (2.1 x 50 mm; particle size 1.8 mm); flow rate 0.5 mL.min^{-1}; mobile phase methanol: water, both with 0.1% formic acid in gradient elution (50% of methanol for 2 min and 50–100% methanol in 3 min and then return to 50% of methanol in 1.5 min, total of 6.5 min); injection volume 4 µL, temperature of 50°C. ESI parameters were capillary voltage 3.5 kV for positive mode; gas temperature 325°C; drying gas 8 L.min^{-1}; fragmentor 175V; skimmer 65V; mass range from 100–1000 *m/z* and no collision energy was used. The TDF calibration curve was prepared in triplicate in water, with concentrations ranging from 0.1 to 125 mg.L^{-1}, followed by filtration (0.22 µm).

Results And Discussion

The analytical method for monitoring the experiments was developed using an aqueous solution of TDF (50.0 mg.L^{-1}), analyzed via HPLC-ESI-Q-TOF/MS. The presence of two peaks (Fig. 2), a main peak (0.7 min) and a residual peak (0.3 min) was observed in the chromatogram. For peak characterization, the isolated ions in the mass spectrum were extracted and the corresponding chemical structure was proposed

based on the exact mass obtained. Thus, the peak at 0.3 min, with m/z 404.13, according to the mass fragmentation pattern described by Kurmi et al. (2015), was assigned to Tenofovir isoproxil protonated monoester (TMF). The peak at 0.7 min, with m/z 520.18 was attributed to protonated tenofovir disoproxil TDF [M + H +]. In the final minutes (after 4 min), no TDF-derived fragment was observed, with the final peaks corresponding to the gradient elution.

Figure 2 Chromatogram of TDF solution in ASM1 medium (50 mg.L^{-1}) showing peaks corresponding to Tenofovir isoproxil monoester (m/z 404.13) and tenofovir disoproxil (m/z 520.18)

According to Kurmi et al. (2015), the tenofovir isoproxil monoester detected in the medium corresponds to the product of partial hydrolysis of TDF and is described as an impurity commonly present in the raw material of the drug (USP, 2011; WHO, 2019).

To carry out the tests, the contribution of abiotic processes to the degradation of TDF in ASM1 medium was evaluated, under controlled conditions (pH, temperature, and light radiation). It was observed in the controls (TDF concentrations 12.5; 25 and 50 mg.L^{-1}) an increase in the peak attributed to the monoester throughout the experiment. At the end of the process (16 days), all the TDF was converted into tenofovir isoproxil monoester, showing that mono-de-esterification can occur spontaneously in the culture medium. Hydrolysis was observed at all TDF concentrations used. ASM1 medium is a mineral medium containing several salts, and the experiment was performed in buffered medium (pH7-8). According to Silva (2014), the hydrolysis of TDF occurs preferably at neutral or alkaline pH, with the molecule being more stable at acidic pH (2 to 3). Thus, the test conditions favor the deesterification of the molecule.

Biodegradation tests were performed using TDF concentrations of 12.5; 25 and 50 mg.L^{-1}. It is important to highlight that this series of concentrations was defined considering that the ability of strains of microorganisms to degrade toxic compounds depends on their intrinsic ability to metabolize the xenobiotic. This property can be constitutive or acquired by microorganisms exposed to conditions considered stressful for the species (Tandeau de Marsac & Houmard, 1993; Zeng et al. 2009). Microbial biochemical pathways can be activated when microorganisms are exposed to critical conditions, such as high concentrations of pollutants, temperature and pH variations (Zeng et al. 2009; Żyszka-Haberecht, Niemczyk, Lipok 2019). In the case of *M. novacekii*, in a previous study, the strain tolerated high concentrations of TDF (EC50% 161.0 mg.L^{-1}) (Silva et al. 2019), allowing the use of drug concentrations of up to 50mg.L^{-1} in this study, configuring a stressful condition to evaluate the metabolization potential of this antiviral by the culture of *M. novacekii*.

During the TDF biodegradation experiments using the *M.* novacekii culture, the drug's mono-desterification was also verified, however, the hydrolysis in the tests was more intense and faster compared to controls (Fig. 3). While in controls total monodesterification occurred over 16 days, in cultures, after 72 h, only TDF residues were detected, indicating that although abiotic factors contribute to hydrolysis, metabolic pathways of microorganisms are probably responsible for accelerating the deesterification process, through extracellular enzymes.

Figure 3 Tenofovir disoproxil and monoester of tenofovir removal during cyanobacteria degradation process in sample of 50 $mg.L^{-1}$

It was verified during the test that the drug and its derivative were gradually extracted from the medium, with a percentage of TDF/TMF removal at the end of the experiment of 91.8% (12.5 $mg.L^{-1}$), 94.1% (25 $mg.L^{-1}$) and 88.7% (50 $mg.L^{-1}$). In controls, no reduction in monoester concentration was observed (Fig. 4). Neither Tenofovir, nor any other metabolite besides TMF, was detected in the culture medium, indicating the probable removal of TMF by the direct action of microorganisms.

Figure 4 Evolution of the peak areas at m/z 520.18 and m/z 404.13 obtained via HPLC/MS during TDF (at concentrations of 12.5, 25.0 and 50.0 $mg.L^{-1}$) biodegradation by *M. novacekii*

To analyze these results, it is necessary to consider that the used culture of *M. novacekii* is unialgal, but not axenic. According to the genetic sequencing performed, the bacterium *Pseudomonas pseudoalcaligenes* was identified in the medium. This is an aerobic, Gram-negative species and its potential to metabolize toxic compounds has been described by several researchers (Igeño et al. 2011; Wallace et al. 2017; Safari et al. 2019). Its production of esterases (Igeño et al. 2011) stands out, including potent arylesterases that give this species the ability to degrade various compounds, including polyesters (Wallace et al. 2017). The production of these esterases may be the accelerating factor of TDF hydrolysis.

The coexistence of cyanobacteria and microalgae with heterotrophic bacteria in the environment has aroused interest in the study of degradation of organic substances, leading to the inclusion of photosynthetic species in microbial consortia. The association of these groups of organisms can be advantageous for reducing energy expenditure due to in situ oxygen production, can also reduce CO_2 emission and increase the production of algal biomass that can be used in the production of various compounds of technological application (Wang et al. 2016; Chan et al. 2022). Thus, several studies of the potential of these associations for the removal of different classes of organic substances have been carried out (Maza-Márquez et al. 2017, da Silva Rodrigues et al. 2020; Wang et al. 2022; Chan et al. 2022).

Phytoplatonic species show similar behavior in the face of stressors (Wiśniewska et al. 2021), which may increase the expression of enzymes degrading organic compounds and other compounds aimed at cellular protection (Touliabah et al. 2022).

In the study of microbial associations for the biodegradation of pollutants, the presence of species of the genus Microcystis is particularly interesting because they are very resistant species to toxic agents due to the protection mechanisms developed throughout the evolutionary process (Tandeau de Marsac and Houmard, 1993). In the case of the genus Microcystis, resistance has been attributed to the characteristics of some species, including those that present a thick mucilaginous layer that surrounds the cells, with diverse functions such as nutrition and protection against dissection and against external agents, in addition to allowing the aggregation of cells in colonies that favor the formation of biofilm (Tandeau de Marsac and Houmard, 1993; Gonçalves, et al. 2017). The role of cell protection by mucilage is highlighted by Pugnetti et al. (2005) who reports that, when exposed to adverse conditions such as the presence of

toxic substances, some species intensify the production of the mucilaginous layer, which behaves like a sponge that absorbs xenobiotics (Leppard, 1995). In general, mucilage is able to biosorb xenobiotics through different interactions, usually weak bonds, without energy consumption and can retain metal ions, natural organic matter and toxic organic substances (Leppard, 1995; Chan et al. 2022). Chan et al. (2022) report that microalgae can biosorb compounds rich in nitrogen, phosphorus, heavy metals, antibiotics, organochlorines, pesticides and azo dyes from aqueous matrices.

The presence of mucilage is one of the factors that facilitate the association with bacteria, since the exopolymers that compose the mucilage can be used for fixation and as a nutritional source by bacteria, offering heterotrophic microorganisms an ideal microenvironment for their growth and metabolism (Shen et al. 2011). Thus, in the associations of cyanobacteria and heterotrophic bacteria, two processes can act in the removal of pollutants, the immobilization of toxic substances by mucilage and microbial degradation by heterotrophic bacteria and by the cyanobacteria themselves (Wang et al. 2022).

In the proposition of cyanobacteria/microalgae and bacteria consortia, although the immobilization by adsorption to mucilage allows the reduction of soluble organic carbon, it is preferable to associate the mechanisms of biodegradation and especially of mineralization of pollutants as they are more effective for the removal of organic compounds. In general, biodegradation occurs through metabolic pathways typical of various microorganisms (bacteria, fungi and algae), which can be expressed under stressful conditions. Biodegradation can lead to partial decomposition of the molecule, generating by-products or total, reducing organic compounds through metabolic processes to their inorganic forms (Chan et al. 2022).

Studies of microalgae/cyanobacterial and bacterial consortia for the purification of drugs from the aquatic environment have been reported with promising results (Gonçalves et al. 2017; Tolboom et al. 2019).The use of these associations for the biodegradation of antimicrobials is highlighted. This is an important characteristic of cyanobacteria and microalgae given the difficulty in obtaining bacterial species tolerant to the biocidal effect of these compounds. Wang et al. (2022) using a consortium of microalgae-heterotrophic bacteria for the degradation of chlortetracycline observed that this drug was initially removed by biosorption, followed by biodegradation. At the end of the experiment, the authors concluded that biosorption alone had a negligible contribution to the drug removal process, which does not mean that biosorption does not have an initial action on drug retention, facilitating the action of enzymes retained in the mucilaginous layer. Biodegradation catalyzed by enzymes secreted by the species present, under stress triggered by the antibiotic, has been identified as the main mechanism of chlortetracycline removal (Wang et al. 2022). These authors observed that the use of microalgae/heterotrophic bacteria cultures for chlortetracycline biodegradation presented better results in terms of drug bioremoval than the respective axenic cultures.

Likewise, in a study of the removal of sulfamethoxazole from the aquatic environment by a consortium of algae and heterotrophic bacteria, da Silva Rodrigues et al. (2020) found that the antibiotic was mainly removed by biodegradation. The bioaccumulation and biosorption of the drug by the microorganisms were negligible. The small contribution of drug biosorption on microalgae cell walls was attributed to the high water solubility of the sulfamethoxazole molecule (da Silva Rodrigues et al. 2020).

In this study using the association of *M. novacekii* and *P. pseudoalcaligenes*, it is likely that the removal of TDF occurs in a similar way to that reported in the degradation of chlortetracycline (Wang et al. 2022) and sulfamethoxazole (da Silva Rodrigues et al. 2020). Possibly in the initial step of the process, monodeesterification occurs by an abiotic process and by the action of extracellular hydrolases from both heterotrophic bacteria and cyanobacteria, since both groups of microorganisms can be esterases producers (Panda and Gowrishankar, 2005; Zheng et al. 2018).

The rapid formation of TMF and its slow removal from the medium, without the presence of other metabolites, suggests that degradation occurs in the intracellular medium and that penetration into cells, whether bacterial or cyanobacteria, is the critical factor for the slow removal of TMF from the medium. Khan et al. (2018) described that during the proliferation of bacterial and microalgae associations, both groups express enzymes such as phosphatase, sulfatase, glucosidase and galactosidase that may be responsible for biodegradation processes of organic compounds. Thus, it is not possible to state which of the groups of microorganisms was responsible for the removal of TMF.

The role of *Microcystis novacekii* in the degradation of TMF is reinforced by the presence in the molecule of phosphate groups, a limiting nutrient for the growth of cyanobacteria. Ren et al. (2017) found that *Microcystis aeruginosa* is able to use dissolved organic phosphate from different chemical compounds to support its growth. In this way, the TMF molecule can provide a source of phosphate for the cyanobacteria.

Although the removal of TDF from the medium occurred with high yield, the presence of residual concentrations of TMF at the end of the experiment was observed for all concentrations tested. At the concentration of 25 $mg.L^{-1}$, the highest removal was obtained, about 94% of the drug and its metabolite, which is a very promising result. Apparently, intra and extracellular conjugated processes occur, requiring further studies to optimize the drug extraction process and its metabolite from the medium.

Even with the good results obtained in this study, it should be noted that tenofovir is an inhibitor of DNA synthesis, and its metabolite TMF is partially active (Brooks et al. 2019), its persistence in the environment can potentially lead to damage to the genetic heritage of exposed species. Thus, the persistence of TMF in the culture medium for more than 15 days is worrying and points to the need for further studies on the biodegradation of this antiviral in order to prevent possible genotoxic actions to other aquatic organisms.

Conclusion

Through this study, excellent results were obtained in the removal of TDF from the culture medium using a culture of *M. novacekii/P. pseudoalkaligenes*. Approximately 94% of the drug and its metabolite were extracted at a concentration of 25 $mg.L^{-1}$. After 16 days, residual concentrations of only one TDF metabolite, tenofovir isoproxil monoester, were detected. This result reinforces the potential of this association for studies on the removal of this drug from more complex matrices. The sustainability of the method, the ease of the technique and the good performance of the culture in removing TDF are advantages that justify the investigation of the potential of this association for environmental uses.

The rapid desterification of TDF in the culture medium releasing the monoester - tenofovir isoproxil was one of the important results of this study. TMF partially maintains the antiviral activity and the persistence of residual concentrations of this compound for more than 16 days in the culture medium is a worrying factor, as it may indicate that this metabolite can accumulate in the environment. In this sense, studies aimed at removing this metabolite are important to prevent exposure of aquatic species to antiviral residues.

Declarations

Conflict of Interest

The authors declare that they have no conflict of interest.

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Figures

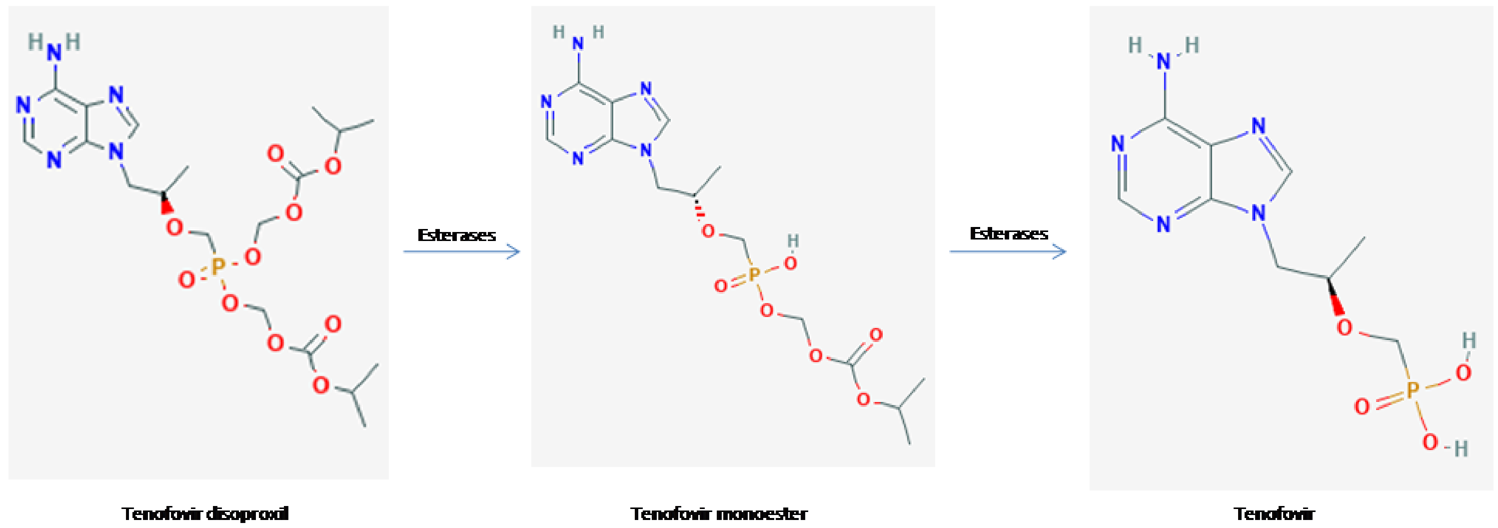

Figure 1

Metabolism of the prodrug tenofovir disoproxil with formation of the de-esterification products: tenofovir monoester and tenofovir

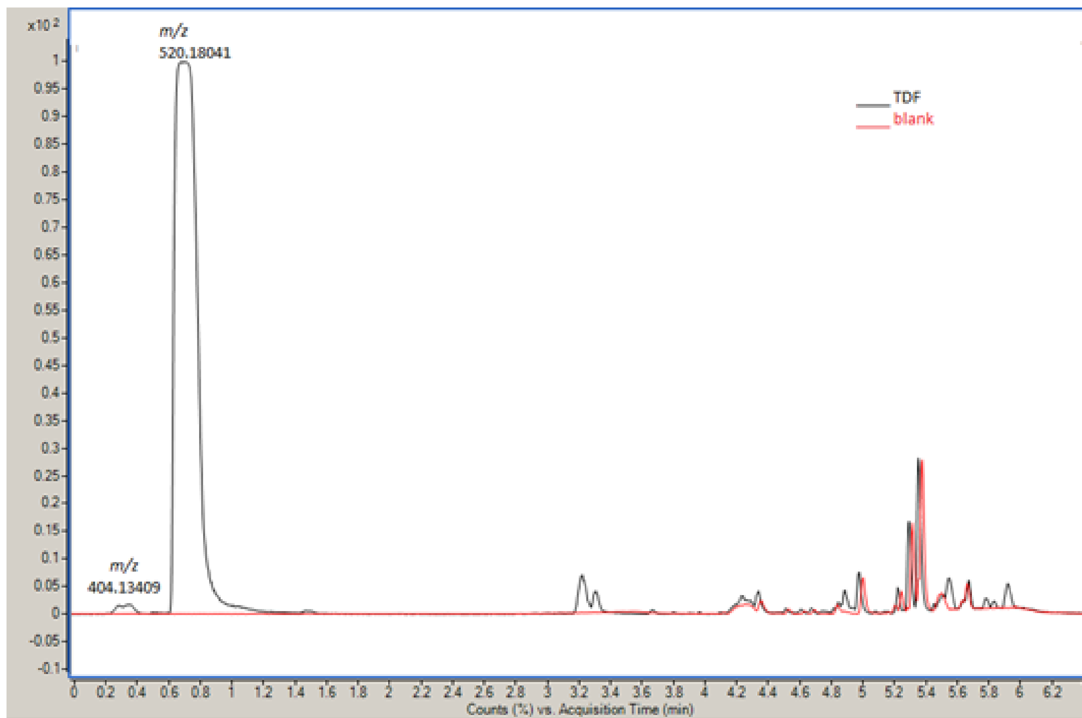

Figure 2

Chromatogram of TDF solution in ASM1 medium (50 mg.L^{-1}) showing peaks corresponding to Tenofovir isoproxil monoester (m/z 404.13) and tenofovir disoproxil (m/z 520.18)

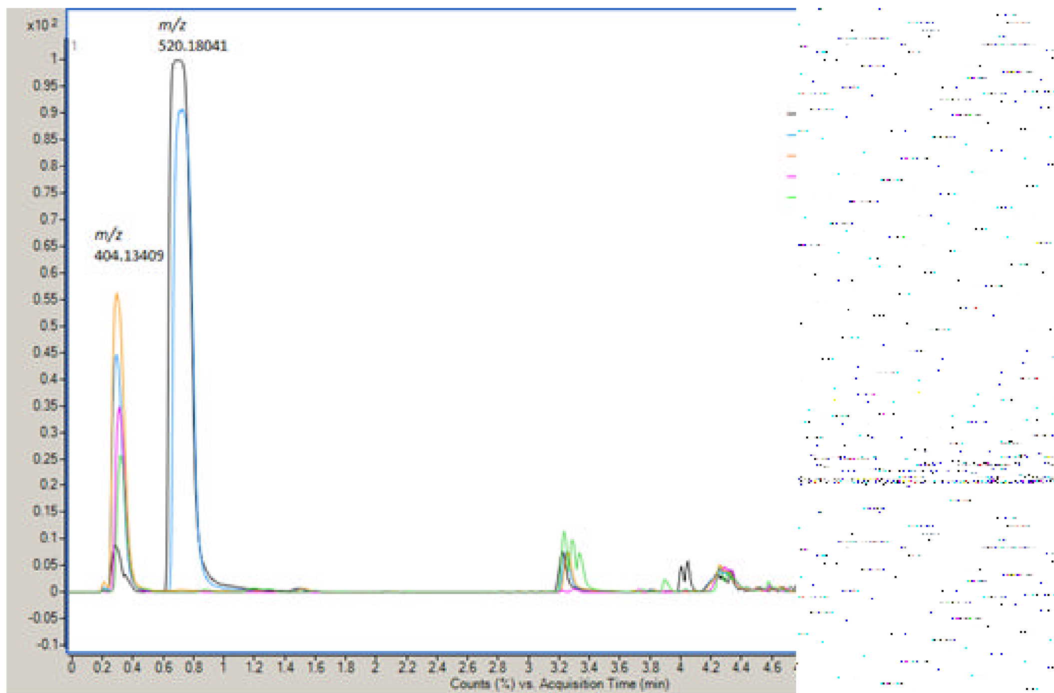

Figure 3

Tenofovir disoproxil and monoester of tenofovir removal during cyanobacteria degradation process in sample of 50 mg.L^{-1}

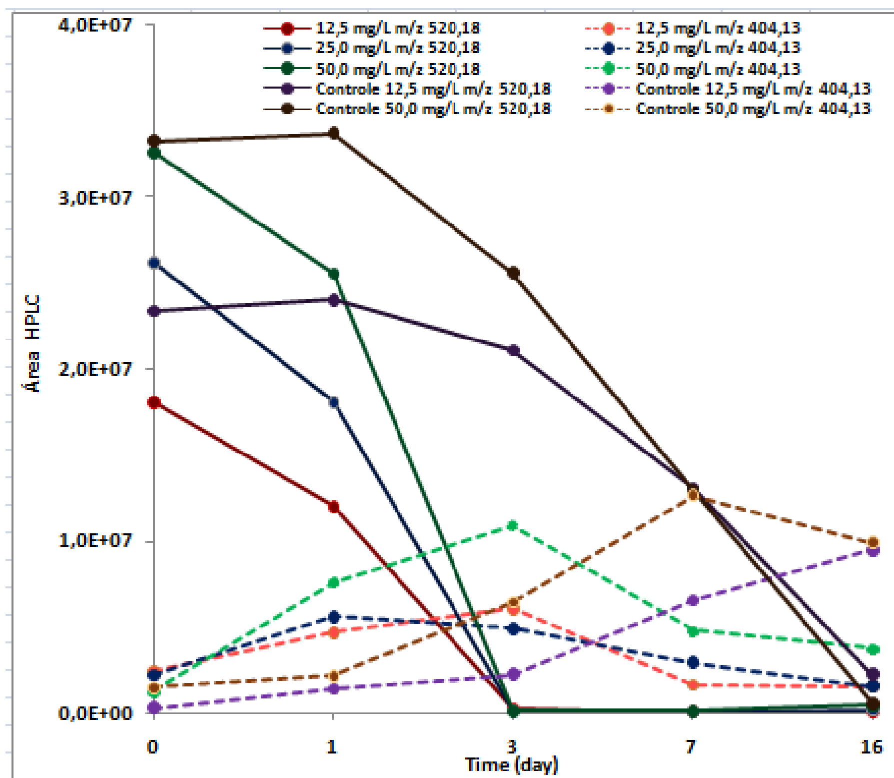

Figure 4

Evolution of the peak areas at m/z 520.18 and m/z 404.13 obtained via HPLC/MS during TDF (at concentrations of 12.5, 25.0 and 50.0 mg.L^{-1}) biodegradation by *M. novacekii*