CHEMICAL ENGINEERING TRANSACTIONS
VOL. 127, 2026
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Laura Capelli, Marzio Invernizzi, Selena Sironi

ISBN 979-12-81206-28-1; **ISSN** 2283-9216
DOI: 10.3303/CET26127049

Adapted Acinetobacter Bereziniae Strain for Efficient Removal of Efavirenz in Pharmaceutical Wastewater: Isolation and Kinetics

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The pervasive administration of antiretroviral drugs (ARVs) for the management of HIV/AIDS has led to their continuous entry into aquatic ecosystems via excretion and the suboptimal removal efficacy of conventional wastewater treatment plants (WWTPs). Efavirenz (EFV), a non-nucleoside reverse transcriptase inhibitor (NNRTI), is prominently detected and recalcitrant in the environment, exhibiting removal efficiencies below 50% in conventional WWTPs due to its hydrophobic nature, halogenation, and structural recalcitrance. This study reports the isolation of a novel *Acinetobacter bereziniae* strain (designated EFV-D1) from mine waste, which exhibited significant potential in the bioremoval of EFV. The isolate was cultured in a mineral salt medium with 50 mg/L EFV as the sole carbon source and identified through 16S rRNA gene sequencing, revealing 99.8% similarity to the *A. bereziniae* species. Batch removal experiments demonstrated that strain EFV-D1 achieved over 50% EFV removal within 24 hours under optimized conditions (pH 7.0, 35°C, agitation at 100rpm). Kinetic modelling followed pseudo–first-order kinetics, yielding a rate constant of 0.044 h^{-1} and an estimated half-life of 15.6 h, confirming efficient substrate utilization. Growth assays further demonstrate that *A. bereziniae* EFV-D1 could tolerate and metabolize EFV concentrations reaching its minimum inhibitory concentration (MIC), indicating resilience and adaptability to pharmaceutical-contaminated wastewater. This investigation establishes *A. bereziniae* EFV-D1 as a potent biocatalyst for the removal of EFV from wastewater, presenting a proof-of-concept of a scalable and cost-effective bioremediation strategy for emerging contaminants of concern. By integrating microbial bioremoval with existing treatment processes, *A. bereziniae* EFV-D1 offers a cost-efficient, environmentally benign, and scalable solution to mitigate the environmental persistence of EFV and analogous pharmaceutical pollutants in wastewater systems.

1. Introduction

Following the initial documentation of HIV, scientific advancements have transformed the once life-threatening disease into one that can be effectively managed through lifelong antiretroviral therapy (ART). ART employs a combination of antiretroviral (ARV) drugs with various mechanisms of action, dose-limiting toxicities, and in vitro application to manage HIV/AIDS (Ward et al., 2021). The adoption of the universal test and start initiative aimed at achieving the UNAIDS 90-90-90 and 95-95-95 targets, which entail that 90% of people living with HIV (PLWHIV) should know their status, 90% of those should be on ART, and 90% of those should achieve viral suppression by 2020, while the 95-95-95 targets were to be achieved by 2023 (UNAIDS, 2023). Expanded ART coverage, combined with high drug excretion rates, has resulted in the release of substantial quantities of ARVs into the environment. Consequently, the drugs have been detected in various locations globally and quantified across a range of environmental matrices, with elevated concentrations observed in countries such as Kenya, South Africa (SA), and Zambia, all situated in the Sub-Saharan African (SSA) region (K'oreje et al., 2012). Recent reviews (K'oreje et al., 2020; Ncube et al., 2018) have documented measured environmental concentrations (MECs) of ARVs in various aquatic environmental systems, which were found to be higher in

Paper Received: 21 April 2026; Revised: 25 June 2026; Accepted: 8 July 2026
Please cite this article as: Ngwenya P., Mpeta M., Tabana L., Tichapondwa S., Chirwa E., 2026, Adapted Acinetobacter Bereziniae Strain for Efficient Removal of Efavirenz in Pharmaceutical Wastewater: Isolation and Kinetics, Chemical Engineering Transactions, 127, 289-294 DOI:10.3303/CET26127049

Africa compared to other regions globally. To mitigate the impacts of pharmaceutical pollution, it is imperative to employ effective wastewater treatment technologies to remove these pollutants from wastewater prior to its discharge into environmental matrices.

In several countries within the SSA region, a regimen consisting of lamivudine (3TC), tenofovir (TDF), and efavirenz (EFV), is usually administered as a combination (Kandel and Walmsley, 2015). Consequently, EFV, with an excretion rate exceeding 60% (Rakhmanina and van den Anker, 2010), is among the most commonly detected ARVs and has been identified in diverse environmental matrices. Detected at concentrations as high as 160 µg/L in Kisumu, Kenya (K'oreje et al., 2020). Sanderson et al., (2004) ranks the toxicity of ARVs towards aquatic biomarkers, including fish, algae, daphnia, and bacteria, among the ten most toxic classes of contaminants. For instance, Almeida et al., (2021) reported that EFV exhibited EC_{50} and NOEC values of 0.026 mg/L and 0.016 mg/L, respectively, following an eight-day exposure to *Ceriodaphnia dubia*. Thus, EFV can be considered extremely toxic to *C. dubia* (Almeida et al., 2021). Additionally, Robson et al., (2017) observed that 0.0103 µg/L of EFV induced significant liver damage and subsequently reduced long-chain triglyceride (Lct) levels in *Oreochromis mossambicus* after 96 hours of exposure, an effect similarly noted in humans (Blas-García et al., 2010). Furthermore, chronic toxicity tests over 28 days, as conducted by Pitso, (2020), demonstrated distinctive renal and liver toxicity in *O. mossambicus*. Omotola et al., (2021) reported that 0.1 mg/L of 3TC resulted in 100% mortality in *Daphnia magna* after 48 hours. Moreover, eight-day chronic toxicity assessments deploying 3TC on *C. dubia* revealed EC_{50} and NOEC values of 1.345 mg/L and 0.625 mg/L, respectively, highlighting the drug's toxicity to daphnia (Almeida et al., 2021).

Conventional wastewater treatment plant (WWTP) processes, which rely predominantly on mixed bacterial consortia optimized for bulk organic matter removal, are ineffective at eliminating EFV. Consequently, previous investigations into EFV removal have focused mainly on advanced oxidation processes (AOPs), particularly UV/TiO_2 photocatalysis (Ncube et al., 2025) and other light-activated catalytic systems (Ngwenya et al., 2025). Although these technologies can attain exceptional removal efficiencies, they are often associated with high energy demands and, in some cases, the formation of potentially more toxic transformation products, thereby limiting their feasibility and scalability, especially in resource-constrained settings. This has prompted growing interest in biological alternatives; however, while microbial degradation has proven effective for other antiretroviral drugs such as abacavir, the literature addressing EFV biodegradation or bioremoval remains sparse. Most reported studies examine EFV removal as part of multicomponent pharmaceutical mixtures and frequently rely on non-bacterial organisms rather than targeted bacterial strains. To illustrate, Tenza and Mahlambi (2025) reported EFV removal efficiencies of 10 – 60% using microalgae over a 10-day period under controlled laboratory conditions. These findings collectively underscore a critical knowledge gap and highlight the need to identify and characterize specialized bacterial strains with the metabolic capability to degrade EFV. Accordingly, the present study seeks to isolate a novel *Acinetobacter bereziniae* strain from mine wastewater and to evaluate its suitability as an EFV-degrading bacterium, thereby advancing strain-specific bioremediation strategies beyond the limitations of conventional WWTP microbial communities.

2. Materials and methods

2.1 Chemicals

The model contaminant, EFV, was obtained from Adcock Ingram. Tryptone Soya Agar (TSA) and Tryptone Soya Broth (TSB) were supplied by Oxoid Ltd. (Basingstoke, Hants, UK). Mineral salts medium (MSM) was prepared as described by Mpeta et al. (2025), supplemented with glucose 10 g/L. Analytical-grade methanol (99%), acetonitrile (99.9%) and hydrochloric acid (HCl, 32%) were obtained as solutions from Sigma-Aldrich. Glacial acetic acid (99%) was procured as a liquid from Glassworld. All reagents were used as received without additional purification, except for NaOH and HCl, which were diluted before they were used for pH adjustment. Deionised water produced by an Elga Purelab Flex 3 water purification system was used as the solvent for all experimental procedures.

2.2 Measurements, values and units

Contaminated soil samples were collected as grab samples from multiple locations, including both historical and freshly processed tailings dumps, at a small-scale gold mine in Chinhoyi, Zimbabwe. Samples were stored in a cooler box at 4 °C until further processing. These samples served as the source of the bacteria investigated in this study.

2.3 Bacterial isolation and storage

Bacteria were isolated from the contaminated soil samples to obtain naturally occurring strains indigenous to these harsh environments. A 0.5 g soil sample (stored at 4 °C) was inoculated into 150 mL of sterile nutrient

broth (TSB) supplemented with 1 mL of an EFV solution at 50 ppm and incubated in a shaking incubator (Scientific Labex Model 353) at 35 ± 2 °C, 120 rpm for 24 h. After 24 h, 0.8 mL of the TSB containing the bacteria was placed in sterilized 2 mL vials before adding 0.2 mL of 50% glycerol solution. Thereafter, the vials were placed in the −70 °C storage chamber. To revive the strain, the frozen vials were removed from the chamber for future use and loops were used to streak contents of the vials onto agar plates. The bacteria were left to grow for 24 h before either being stored for future use or inoculated into fresh TSB for growth. For the removal experiments, the bacteria inoculated in fresh TSB grew for 24 h to their stationary phase. Thereafter, it was harvested and concentrated via centrifugation and the resultant pellet was added to the flasks for ARV removal.

2.4 Identification and characterization of functional isolates

Characterization and taxonomic identification of bacterial isolates were carried out by 16S rRNA gene sequencing at a commercial facility (Inqaba Biotechnical Industries (Pty) Ltd., Pretoria, South Africa). Genomic DNA was extracted from the cultures using the Quick-DNATM Fungal/Bacterial Miniprep Kit (Zymo Research, Catalogue No. D6005). Amplification of the 16S rRNA gene was performed using universal bacterial primers 16S-27F (AGAGTTTGATCMTGGCTCAG) and 16S-1492R (CGGTTACCTTGTTACGACTT).

2.5 Bacterial growth measurements

Growth parameters for *A. bereziniae* were set at a temperature range of 35 – 40°C, neutral pH and agitation at 100 rpm as these are documented in literature as optimal conditions for bacterial growth (Mpeta et al., 2026). The optical density (OD) of the bacterial culture was measured at a wavelength of 600 nm (OD_{600}), to estimate the concentration of bacterial cells in the liquid culture. Aliquots were taken at hourly intervals and OD_{600} was measured using a VWR, UV–Vis spectrophotometer.

2.6 Chemical analysis methods

The surface morphology of A. bereziniae was investigated by Scanning Electron Microscopy (SEM) Zeiss Ultra Plus Instrument. While the interfacial interactions and bacterial ultrastructure were analysed using a Field Emission Transmission Electron Microscopy (JEOL JEM-2100F TEM). To quantify EFV removal aliquots of EFV-containing samples were also collected at hourly intervals, filtered, and analyzed by high-performance liquid chromatography (HPLC) using a Waters Alliance 2695 system equipped with a UV–Vis detector and autosampler. Chromatographic separation was achieved on a Waters PAH C18 column. Data acquisition and processing were performed using Empower software. Isocratic elution was carried out at 35 °C with a mobile phase consisting of 65% (v/v) acetonitrile and 35% (v/v) buffer solution adjusted to pH 3.5. Thereafter, removal efficiency was computed using Equation 1 below:

- $Removal\ efficiency = \frac{C_0 - C_t}{C_0} \times 100\ \%$ (1)

3. Results and discussion

3.1 Isolation, identification and characterization of *A. bereziniae*

The characterization of the bacterial strains was conducted on colonies grown on tryptone soy agar by Inqaba Biotechnical Industries Private Limited, which gave the photographic image of the 16S target region, BLAST results and 16S report (Table 1). The bacteria strains were identified as *Acinetobacter bereziniae (A. bereziniae)*.

Table 1: Bacterial identification results (16S) showing similarity between the sequence queried and the biological sequences within NCBI database.

Name of sample	M.M CS 3 03/02/2025
Request ID	UBGJMD4G016
Predicted organism	*Acinetobacter bereziniae*
GenBank accession	NR_117625.1
E-Value	0
HSP Length	1437bp
% Identity	99.72%

SEM imagery of *A. berezinae* (Figure 1a) revealed short, plump rod-shaped bacteria, coccobacilli with a textured morphology similar to rod to coccus shaped observed by Nemec et al. (2010) in the *Acinetobacter spp*. While TEM (Figure 1b) depicts individual ultrathin cells with dense cytoplasmic granules plausibly representing glycogen storage, predominantly reported in nutrient -constrained Acinetobacter cultures (Koning et al., 2023).

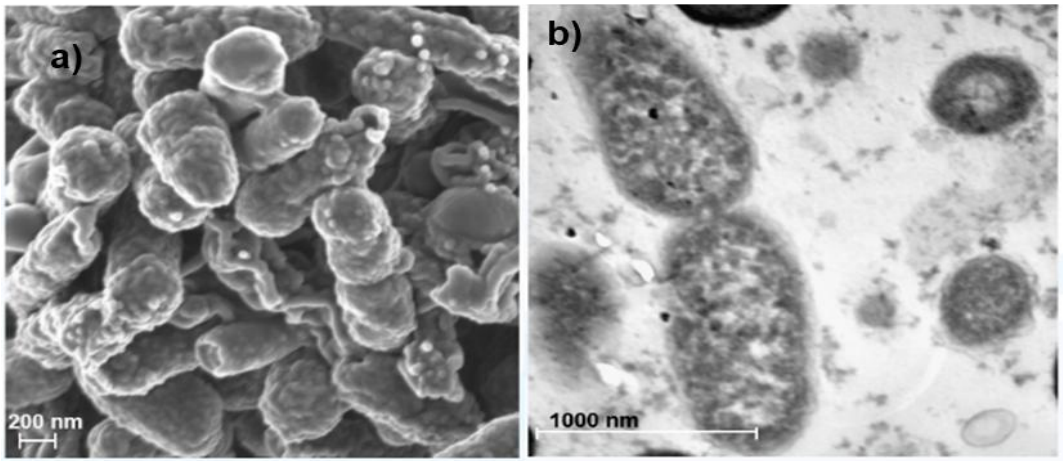

Figure 1: a) SEM and b) TEM images showing A. berezinae morphology and size

3.2 Bacterial growth in the presence of EFV

Growth of A. bereziniae in the presence of EFV was monitored via OD600 over 24 h, exhibiting a multiphasic profile indicative of adaptive response, as seen in Figure 2a. OD600 increased rapidly from ≈ 0.1 to 1.3 within the first 2 h, likely reflecting initial utilization of residual medium nutrients and inherent EFV tolerance, consistent with the metabolic versatility of Acinetobacter spp. (Wang et al., 2018). This was followed by a sharp decline to near zero by 4 h, attributable to EFV-mediated growth inhibition or cell lysis, as observed in other bacteria such as Enterococcus faecalis (Ray et al., 2021). A prolonged lag phase ensued until ≈ 12 h, after which regrowth occurred, reaching OD600 ≈ 0.9 by 24 h. This delayed recovery suggests metabolic adaptation enabling partial utilization of EFV or its transformation products as carbon sources, analogous to fluoroquinolone biotransformation in A. baumannii involving aromatic ring cleavage and substitution (Shaker et al., 2022). These findings underscore the catabolic potential of A. bereziniae for pharmaceutical bioremediation.

3.3 Bioremoval of EFV

Acinetobacter bereziniae exhibited effective removal of EFV, achieving >50% removal within 24 h under optimized experimental conditions (Figure 2b). The temporal removal profile shows a progressive and sustained decrease in EFV concentration, demonstrating the capacity of *A. bereziniae* to mediate the biological transformation of this compound. During the initial phase (0–4 h), the bacteria preferentially utilize glucose, a readily assimilable carbon source present in the MSM or other organic constituents of the medium, thereby supporting rapid initial growth. Upon depletion of this primary substrate, a transient growth arrest is observed (4–5 h), corresponding to the period required for the induction of enzymes and metabolic pathways necessary for the utilization of the secondary, less preferred substrate, namely EFV and/or its early transformation products. Following this adaptation phase (5–24 h), bacterial growth resumes on EFV (or its metabolites), which coincides with active removal of the parent compound.

This metabolic behaviour aligns with the well-documented catabolic versatility of *Acinetobacter spp*., which are known to harbour an extensive repertoire of oxygenases and hydrolases capable of cometabolically degrading structurally complex and hydrophobic organic contaminants (Wang et al., 2018). The initially steeper decline in EFV concentration suggests a phase of rapid microbial adaptation and preferential transformation of more labile molecular moieties, whereas the subsequent plateau phase likely reflects the persistence of halogenated and fluorinated functional groups that are inherently resistant to enzymatic cleavage. Similar removal patterns have been reported in previous studies, with EFV removal efficiencies ranging from approximately 98% over 10 days in moving bed biofilm reactors (Mokgone et al., 2022) to less than 50% within 4 h using laccase-mediated oxidation (Malati et al., 2023) thereby highlighting the kinetic constraints associated with both enzymatic and microbial degradation pathways.

The removal of EFV by *A. bereziniae* followed pseudo–first–order kinetics, indicating that the removal rate was primarily controlled by substrate availability at the relatively low EFV concentrations employed (Çeçen and Gül, 2021). The estimated rate constant of 0.044 h^{-1} and the corresponding half-life of 15.6 h are indicative of moderate yet sustained removal, which is consistent with the recognized recalcitrance of EFV arising from its aromatic and halogenated structural features. Collectively, these results confirm the predictable kinetic behaviour and degradation performance of *A. bereziniae* and support its potential application in biologically enhanced wastewater treatment systems for the removal of ARV drugs.

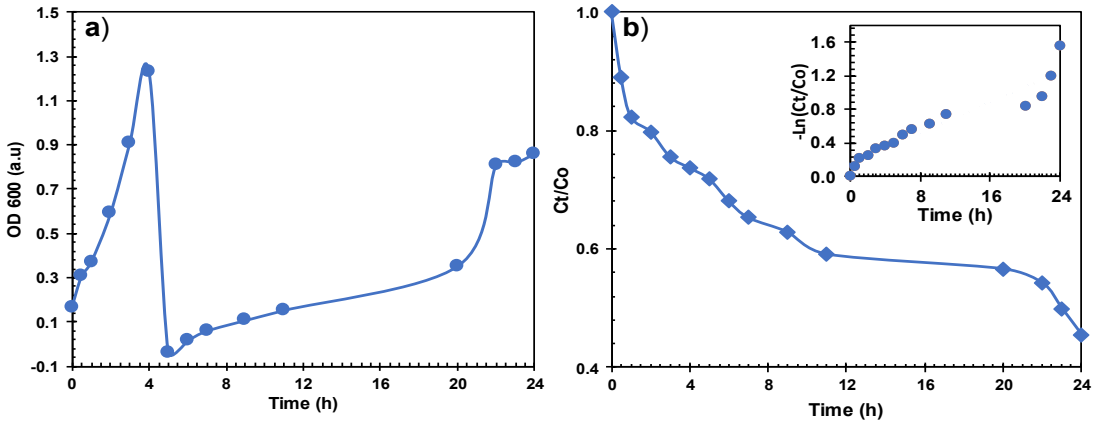

Figure 2: Optical density at 600nm showing multiphasic growth of A. berezinae in 24 hr during the removal of EFV a) and b) EFV removal mediated by A. bereziniae during 24 h incubation.

4. Conclusions

The removal of EFV by A. berezinae displays moderate efficiency, with a removal rate > 50%. Growth in an EFV-laced medium resulted in a diauxic growth profile, consistent with EFV functioning as a non-preferred primary carbon source. Although the observed removal is ascribed to biological activity, additional investigations, including metabolite identification by LC–MS, are required to conclusively delineate the removal pathway. Nonetheless, the partial transformation of EFV likely yields assimilable intermediate metabolites following the initial enzymatic attack, thereby providing a secondary substrate that sustains subsequent growth. Future work will focus on elucidating the underlying removal mechanisms, specifically to determine whether EFV elimination occurs predominantly through adsorption to the cell surface, biosorption, or intracellular accumulation. In parallel, the identification and characterization of intermediate compounds formed during EFV transformation, as well as the enzymes implicated in EFV removal, will be pursued.

References

Almeida, L.C., Mattos, A.C., Dinamarco, C.P.G., Figueiredo, N.G., Bila, D.M., 2021. Chronic toxicity and environmental risk assessment of antivirals in Ceriodaphnia dubia and Raphidocelis subcapitata. Water Sci. Technol. 84, 1623–1634.

Blas-García, A., Apostolova, N., Ballesteros, D., Monleón, D., Morales, J.M., Rocha, M., Victor, V.M., Esplugues, J.V., 2010. Inhibition of Mitochondrial Function by Efavirenz Increases Lipid Content in Hepatic Cells. Hepatology 52, 115–125.

Çeçen, F. and Gül, G., 2021. Biodegradation of five pharmaceuticals: estimation by predictive models and comparison with activated sludge data. International Journal of Environmental Science and Technology, 18(2), pp.327-340.

Koning, R.I., de Breij, A., Oostergetel, G.T., Nibbering, P.H., Koster, A.J. and Dijkshoorn, L., 2013. Cryo-electron tomography analysis of membrane vesicles from Acinetobacter baumannii ATCC19606T. Research in microbiology, 164(5), pp.397-405.

Kandel, C., Walmsley, S., 2015. Dolutegravir – a review of the pharmacology, efficacy, and safety in the treatment of HIV. Drug Des. Devel. Ther. 3547. https://doi.org/10.2147/DDDT.S84850

K'oreje, K.O., Demeestere, K., De Wispelaere, P., Vergeynst, L., Dewulf, J., Van Langenhove, H., 2012. From multi-residue screening to target analysis of pharmaceuticals in water: Development of a new approach based on magnetic sector mass spectrometry and application in the Nairobi River basin, Kenya. Sci. Total Environ. 437, 153–164.

K'oreje, K.O., Okoth, M., Van Langenhove, H., Demeestere, K., 2020. Occurrence and treatment of contaminants of emerging concern in the African aquatic environment: Literature review and a look ahead. J. Environ. Manage. 254, 109752.

Malati, M.L., Abrahams, A., Chauke, V., Seopela, M.P. and Dlamini, L.N., 2023. Monitoring and optimizing the biocatalytic degradation of efavirenz with immobilized Trametes versicolor laccase on Ti2NTx MXene: A lab-scale wastewater treatment plant process. *J. Environ. Chem. Eng.*, *11*(5), p.110420.

Mokgope, H., Leudjo Taka, A., Klink, M.J., Pakade, V.E. and Walmsley, T., 2022. Quantification of some ARVs' removal efficiency from wastewater using a moving bed biofilm reactor. *Water Sci & Tech.*, *86*, 2928-2942.

Mpeta, M., Tendenedzai, J.T., Tichapondwa, S.M. and Chirwa, E.M., 2025. Effect of Cyanide as a Nutrient Source on bacteria, Bacillus Cereus, for use in Bioleaching of Gold Tailings. *Chem. Eng. Tran.*, *117*, pp.1075-1080.

Mpeta, M., Tendenedzai, J.T., Tichapondwa, S.M. and Chirwa, E.M., 2026. Performance evaluation of gold (III) bio-reduction by bacterial strains and a consortium isolated from gold mine effluent. Minerals Engineering, 235, p.109797.

Ncube, P., Zvinowanda, C., Ntuli, F., 2025. Photocatalytic Degradation of Emtricitabine, Lamivudine, and Tenofovir in Water Using the UV/TiO2/H2O2 Hybrid System. Int. J. Environ. Res. 19, 91.

Ncube, S., Madikizela, L.M., Chimuka, L., Nindi, M.M., 2018. Environmental fate and ecotoxicological effects of antiretrovirals: A current global status and future perspectives. Water Res. 145, 231–247.

Nemec, Alexandr, Martin Musílek, Ondrej Šedo, Thierry De Baere, Martina Maixnerova, Tanny JK van der Reijden, Zbyněk Zdráhal, Mario Vaneechoutte, and Lenie Dijkshoorn. "Acinetobacter bereziniae sp. nov. and Acinetobacter guillouiae sp. nov., to accommodate Acinetobacter genomic species 10 and 11, respectively." International journal of syst.and evol. microb. 60, no. 4 (2010): 896-903.

Ngumba, E., Gachanja, A., Tuhkanen, T., 2016. Occurrence of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya. Sci. Total Environ. 539, 206–213.

Ngwenya, P., Tabana, L.S., Tichapondwa, S.M., Chirwa, E.M.N., 2025. Occurrence, Ecotoxicity, and Photocatalytic Remediation of Antiretroviral Drugs in Global Surface Water Matrices. Catalysts 15, 381.

Omotola, E.O., Genthe, B., Ndlela, L., Olatunji, O.S., 2021. Environmental Risk Characterization of an Antiretroviral (ARV) Lamivudine in Ecosystems. Int. J. Environ. Res. Public. Health 18, 8358.

Pitso, K.M., 2020. Mozambique tilapia Oreochromis mossambicus. University of Johannesburg, Johannesburg.

Rakhmanina, N.Y., van den Anker, J.N., 2010. Efavirenz in the therapy of HIV infection. Expert Opin. Drug Metab. Toxicol. 6, 95–103.

Robson, L., Barnhoorn, I.E.J., Wagenaar, G.M., 2017. The potential effects of efavirenz on Oreochromis mossambicus after acute exposure. Environ. Toxicol. Pharmacol. 56, 225–232.

Ray, S., Narayanan, A., Giske, C.G., Neogi, U., Sönnerborg, A. and Nowak, P., 2020. Altered gut microbiome under antiretroviral therapy: impact of efavirenz and zidovudine. ACS infectious diseases, 7(5), pp.1104-1115.

Sanderson, H., Johnson, D.J., Reitsma, T., Brain, R.A., Solomon, K.R., 2004. Ranking and prioritization of environmental risks of pharmaceuticals in surface waters. Regul. Toxicol. Pharmacol. 39, 158–183.

Schwarzer, R., Gramatica, A., Greene, W.C., 2020. Reduce and Control: A Combinatorial Strategy for Achieving Sustained HIV Remissions in the Absence of Antiretroviral Therapy. Viruses 12, 188.

Shaker, R.A.E., Nagy, Y.I., Adly, M.E., Khattab, R.A. and Ragab, Y.M., 2022. Acinetobacter baumannii, Klebsiella pneumoniae and Elizabethkingia miricola isolated from wastewater have biodegradable activity against fluoroquinolone. World Journal of Microbiology and Biotechnology, 38(11), p.187.

Tenza, N.P., Mahlambi, P.N., 2025. An Innovative Bioremediation Approach for the Removal of Antibiotic and Antiretroviral Drug Contaminants From Wastewater Using Chlorella spp. Water Environ. J. 39, 548–560.

UNAIDS, 2023. Understanding measures of progress towards the 95–95–95 HIV testing, treatment and viral suppression targets.

Wang, S., Hu, Y. and Wang, J., 2018. Biodegradation of typical pharmaceutical compounds by a novel strain Acinetobacter sp. Journal of environmental management, 217, pp.240-246.

Ward, A., Mota, T., Jones, B., 2021. Immunological approaches to HIV cure. Semin. Immunol. 51, 101412