Environmental Research and Planetary Health

Research Article
Volume 2(2), 2026, 123–135
https://doi.org/10.53623/erph.v2i2.1327

Biodegradation of Trimethoprim by a Bacterial Consortium Enriched from a Sewage Treatment Plant

Tristan Nilsson[1], Bliss Broussard[1], Darcey Wayment[2], Himanshu Raje[1]*, Ramaraj Boopathy[1]*, Achmad Syafiuddin[3]

[1]Department of Biological Sciences, Nicholls State University, Thibodaux, Louisiana 70310, United States
[2]Department of Chemistry and Physical Sciences, Nicholls State University, Thibodaux, Louisiana 70310, United States
[3]Center for Industrial and Environmental Health Studies, Universitas Indonesia, 12345 Depok, Indonesia

*Correspondences: himanshu.raje@nicholls.edu; ramaraj.boopathy@nicholls.edu

SUBMITTED: 6 August 2026; REVISED: 28 September 2026; ACCEPTED: 28 September 2026

ABSTRACT: Antibiotic resistance is a persistent public health concern. The excessive use of prescription antibiotics in medical and animal husbandry practices has resulted in potentially harmful levels of antibiotic residues in the environment. Antibiotics released through excreta and industrial effluents exert selective pressure on environmental bacteria, promoting the proliferation and accumulation of antibiotic-resistant bacteria. Diverse microbial communities exhibiting antibiotic resistance may also serve as effective antibiotic degraders. Sewage treatment plants are among the environments most conducive to the development of microbial communities capable of degrading antibiotics. This study focused on Louisiana because it ranks among the five U.S. states with the highest antibiotic use. Trimethoprim (TMP) is one of the commonly prescribed antibiotics. The purpose of this study was to investigate the presence of TMP-degrading microbial communities in a small community sewage treatment plant. Sludge was collected from the anaerobic digester at the Thibodaux sewage treatment plant, and a microbial consortium was subsequently enriched. The consortium was cultivated with TMP under various growth conditions. TMP degradation was analyzed using high-performance liquid chromatography and mass spectrometry. The results showed that the consortium was capable of degrading TMP under co-metabolic conditions, with 4-desmethyl trimethoprim identified as a major metabolite. The *dfrA12* and *dfrA5* genes, which are associated with TMP resistance, were detected in DNA isolated from the consortium.

KEYWORDS: Wastewater treatment plant; biodegradation; trimethoprim; antibiotics

1. Introduction

Antibiotic resistance is a prevalent public health issue. The development of antibiotic resistance has accelerated as a consequence of the increased use of antibiotics. An average increase of approximately 30% in antibiotic misuse was reported between 2000 and 2021 [1]. Global antibiotic consumption is expected to increase by 15% by 2030 [2]. Increased antibiotic use exposes bacteria to antimicrobial agents and consequently selects for antibiotic-resistant bacteria (ARB) [3]. Under selective conditions, resistant bacteria can rapidly outcompete non-

resistant microorganisms, even at relatively low antibiotic concentrations [4]. Antibiotic resistance can also develop through the horizontal transfer of genetic material carrying antibiotic resistance genes (ARGs) [5].

According to the Antibiotic Resistance Threats Report by the Centers for Disease Control and Prevention (CDC), the spread of antibiotic resistance is associated with the overuse and misuse of antibiotics, improper disposal, poor hygiene, animal and agricultural practices, wastewater and sewage, hospital effluents, and healthcare-associated infections [6]. Antibiotics used in human and veterinary medicine can be discharged into the environment through urine and feces in biologically active forms, thereby contaminating soil, water, and plants [7]. The presence of antibiotics, ARB, and ARGs in local waters in southeastern Louisiana has previously been reported [8–10]. Although antibiotics are essential for treating bacterial infections, their improper disposal into aquatic systems and inadequate wastewater treatment may facilitate their environmental dissemination. Bacteria present in sewage treatment plants have been shown to harbor ARGs, suggesting that exposure to antibiotics may create selective conditions that favor resistant populations and facilitate the exchange of genetic material [9]. Following administration, antibiotics are metabolized to varying degrees and may subsequently enter wastewater treatment plants through human excretion [9]. Garcia et al. [11] reviewed the occurrence of organic contaminants, including antibiotics, in the environment, particularly in wetlands, and discussed approaches being investigated for their removal. Because these contaminants possess diverse and complex chemical structures, their removal and degradation mechanisms can vary considerably.

One of the frequently prescribed antibiotics in the United States is trimethoprim (TMP). TMP is used to treat bacterial infections, including urinary tract infections, shigellosis, ear infections, and bronchitis [6]. In 2017, approximately 18.7 million TMP prescriptions were issued in the United States, corresponding to approximately 57 prescriptions per 1,000 people [12]. Trimethoprim is a 2,4-diaminopyrimidine derivative containing a 2,4,5-trimethoxybenzyl group and is a bacteriostatic antibiotic that inhibits dihydrofolate reductase (DHFR). The *folA* gene encodes the bacterial DHFR enzyme, which has a high binding affinity for TMP [13]. Because DHFR is essential for folate metabolism and, consequently, the synthesis of DNA, RNA, and proteins, its inhibition results in bacteriostasis [13]. Although bacterial and human DHFR perform similar biological functions, these orthologous enzymes differ structurally in their ligand-binding sites [15]. Consequently, TMP exhibits substantially greater inhibitory activity against bacterial DHFR than against human DHFR. Bacteria can develop resistance to TMP through genes encoding Type A dihydrofolate reductase (*dfrA*) or Type B dihydrofolate reductase (*dfrB*) [16, 17]. *dfrA* genes are commonly associated with mobile gene cassettes and encode DHFR variants homologous to the *folA*-encoded enzyme but with a lower binding affinity for TMP because of structural alterations in the binding site. In contrast, *dfrB* genes encode a structurally distinct homotetrameric DHFR with low susceptibility to TMP while retaining the ability to reduce dihydrofolate to tetrahydrofolate [18]. These resistance determinants therefore facilitate microbial survival in the presence of TMP.

Among the mechanisms employed by bacteria to reduce antibiotic toxicity, some microorganisms can transform or degrade antibiotics, potentially decreasing their inhibitory effects. Through enzymatic transformation, microorganisms may modify antibiotic structures, and in some cases, the resulting compounds may be further utilized during microbial metabolism. However, several environmental factors can influence the feasibility and rate of

antibiotic biodegradation. Previous studies have demonstrated the ability of microorganisms to degrade TMP under specific environmental conditions, including different pH and redox conditions. pH can influence the ionic state and bioavailability of antibiotics and can also affect microbial activity and the enzymatic pathways involved in their degradation. The extent of TMP biodegradation and the formation of transformation products may also depend on the initial TMP concentration. In wastewater treatment systems, lower TMP concentrations (starting at approximately 5 µg/L) were reported to undergo demethylation and subsequent transformation into 2,4-diaminopyrimidine-5-carboxylic acid through intermediate transformation products, whereas higher concentrations (approximately 500 µg/L) resulted in different patterns of transformation-product formation [19]. Potential intermediate products have included 2,4-diaminopyrimidine and 1,2,3-trimethoxybenzene [20]. TMP degradation of approximately 83% has also been reported in a sequencing batch reactor [19].

Varying degrees of TMP degradation, ranging from approximately 20% to more than 90%, have been observed in biological wastewater treatment systems [21]. Biodegradation has been reported in activated sludge, where microbial communities contribute to the removal or transformation of organic matter, nitrogen, phosphorus, and various micropollutants [22, 23]. Wastewater treatment plants may also employ anaerobic digesters to stabilize activated sludge and convert organic matter into methane, carbon dioxide, and intermediate compounds through hydrolysis, acidogenesis, acetogenesis, and methanogenesis [24]. Complex organic compounds, including some antibiotics, may initially undergo transformation into simpler compounds before further biodegradation or mineralization [25].

The South Thibodaux Sewage Treatment Plant, located in Thibodaux, Louisiana, serves more than 15,000 people and treats up to five million gallons of raw sewage [9]. Antibiotic-resistant bacteria have previously been detected in both raw and treated sewage from this treatment plant [26]. Treated wastewater containing ARB, residual antibiotics, and ARGs may subsequently be discharged into surrounding wetlands, potentially providing a pathway for their dissemination into receiving environments [27]. Wetland plants may take up water containing antibiotic residues or transformation products, potentially contributing to their environmental transport. Antibiotics, ARB, and ARGs may also persist in wastewater and sludge during treatment. Following stabilization, sludge may be transferred to drying beds and subsequently used as biosolids for land application, creating another potential pathway for the environmental dissemination of antibiotic residues and resistance determinants [28].

Therefore, investigating TMP biodegradation by microbial communities derived from anaerobic digester sludge, together with the occurrence of TMP resistance genes, is important for understanding the environmental fate of TMP and its relationship with antimicrobial resistance. This study aimed to determine the conditions that promote TMP biodegradation by an enriched microbial consortium obtained from anaerobic digester sludge, evaluate the effects of TMP on bacterial growth, identify TMP transformation products, and detect genes associated with TMP resistance.

2. Materials and Methods

2.1. Sample Collection and Enrichment of Bacterial Consortium.

Anaerobic digester sludge was collected from the Thibodaux Sewage Treatment Plant using sterile collection bottles and stored at 4 °C until further use. Basic mineral salts (BMS) medium

was prepared using 1 L of deionized water containing 3.5 g of dipotassium phosphate, 1.5 g of monopotassium phosphate, 0.1 g of magnesium sulfate, 0.1 g of sodium chloride, 0.25 g of ammonium sulfate, and 0.1 g of yeast extract. An aliquot of 100 mL of BMS medium containing 100 ppm TMP was inoculated with 5 g of the sludge sample. The culture bottles were incubated at 37 °C for seven days. A second enrichment culture was prepared by transferring 5 mL of inoculum from the first enrichment into 100 mL of fresh BMS medium containing 100 ppm TMP. The culture was incubated until an optical density (OD_{600}) of 0.5 was reached. This second enrichment culture was subsequently used as the inoculum for further experiments.

2.2. TMP Tolerance Test.

A TMP-resistant microbial consortium was enriched as described in Section 2.1. An aliquot (1 mL) of the consortium was inoculated into 20 mL of BMS medium containing 500 ppm glucose and 100 ppm TMP. This treatment was designated as the co-metabolic condition. For the no-TMP condition, 1 mL of the same consortium was inoculated into 20 mL of BMS medium containing 500 ppm glucose without TMP. Additionally, 1 mL of the consortium was inoculated into 20 mL of BMS medium containing 100 ppm TMP without glucose. This treatment was designated as the TMP-only condition. An uninoculated medium containing 100 ppm TMP and 500 ppm glucose served as the abiotic control. The tolerance of the consortium to TMP was further evaluated at TMP concentrations of 0, 100, 200, 300, 400, and 500 ppm in BMS medium under co-metabolic conditions. All treatments contained 500 ppm glucose in 20 mL of BMS medium. Incubations were performed at 37 °C in a shaking incubator (Thermo Fisher Scientific, St. Louis, MO, USA) at 100 rpm. All experiments were conducted in triplicate. Consortium growth was monitored daily for 14 days by measuring the optical density at 600 nm (OD_{600}) using a BIOLOG turbidometer. Total organic carbon (TOC) was analyzed using a YSI 1080 Combustion TOC Analyzer. A 2 mL sample was combusted at 680 °C, and the total carbon content was determined based on the amount of CO_2 generated during combustion.

2.3. TMP Analysis.

TMP concentrations in the samples were determined using a Hitachi high-performance liquid chromatography (HPLC) system (LaChrom Elite L-2310, Hitachi, Dallas, TX, USA) equipped with a UV–Vis detector (L-2420), column oven (L-2350), and autosampler (L-2200U). Samples (5 µl) were injected onto a C18 ZORBAX Eclipse PAH column (3.5 µm, 4.6 × 100 mm; Agilent, Santa Clara, CA, USA). Gradient elution was performed from 8:92 (v/v) acetonitrile$_2$O containing 0.1% formic acid to 33:67 (v/v) acetonitrile$_2$O containing 0.1% formic acid over 14 min. The flow rate was maintained at 1.0 ml/min, and the column temperature was maintained at 30 °C. TMP was detected by UV absorption at 285 nm. A calibration curve was prepared using TMP standards at concentrations of 0, 25, 50, 75, and 100 ppm in deionized water. Mass spectrometric analysis of the culture samples was performed using an LC–MS/MS system consisting of an Agilent 1260 Infinity II HPLC coupled to an Agilent 6470B triple-quadrupole mass spectrometer (LC/TQ) equipped with a Jet Stream™ electrospray ionization (ESI) source. Chromatographic separation was achieved using a ZORBAX Eclipse Plus C18 column (2.1 × 50 mm, 1.8 µm) with gradient elution. Mobile phase

A consisted of water containing 0.1% formic acid, while mobile phase B consisted of acetonitrile. The gradient was programmed from 5% to 50% B over 0–6 min and maintained at 50% B from 6 to 7 min. The column temperature was maintained at 35 °C, and the flow rate was 0.4 ml/min. The mass spectrometer was operated in positive electrospray ionization mode with the following parameters: capillary voltage, 3,500 V; nozzle voltage, 0 V; nitrogen nebulizer pressure, 40 psi; gas temperature, 250 °C; sheath gas temperature, 325 °C; gas flow rate, 7 l/min; and sheath gas flow rate, 11 l/min. The fragmentor voltage was set at 135 V, and data were collected in MS/MS mode.

2.4. Antibiotic Resistance Analysis.

An aliquot (5 mL) of the microbial consortium was inoculated into BMS medium containing 100 ppm TMP and 500 ppm glucose and incubated for three days at 37 °C. DNA was extracted from the resulting microbial biomass using the Mo Bio PowerSoil DNA Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. *dfr* genes were detected using PCR based on the method described by Grape et al. [15]. PCR amplification was performed using primers obtained from Sigma-Aldrich Co. Each PCR reaction contained 5 µL of extracted DNA, 16 µL of deionized water, 25 µL of GoTaq Green Master Mix, and 2 µL each of the forward and reverse primers. Amplification was performed using an Applied Biosystems thermal cycler for 30 cycles. The amplification conditions for the *dfr* genes and 16S rRNA gene consisted of denaturation at 94 °C for 30 s, annealing at 53 °C for 1 min, and extension at 72 °C for 30 s, followed by a final extension at 72 °C for 7 min. The amplified PCR products were separated by electrophoresis using a 1.8% agarose gel containing ethidium bromide in 1× Tris-acetate-EDTA (TAE) buffer. An aliquot of 10 µL of each PCR product was loaded into the gel wells, and a 100-bp DNA ladder was used to estimate the sizes of the amplified fragments. Electrophoresis was performed using a Bio-Rad PowerPac Basic system at 120 V for 45 min. DNA bands were visualized under UV illumination using an Alpha Innotech FluorChem FC2 imaging system, and images were captured using FluorChem FC2 software. Amplification of the 16S rRNA gene was used to confirm the presence of bacterial DNA, whereas positive amplification of the targeted *dfr* genes indicated the presence of TMP resistance determinants.

2.5. Statistical Analysis.

All experiments were conducted in triplicate and included an abiotic control where applicable. Statistical significance was evaluated at a Type I error rate of $\alpha = 0.05$. Repeated-measures analysis of variance (ANOVA) and paired-samples *t*-tests were used to evaluate differences in bacterial growth among the experimental conditions.

3. Results and Discussion

3.1. Trimethoprim Consortium Condition and Concentration Testing.

The bacterial consortium demonstrated growth under all tested trimethoprim (TMP) conditions (Figure 1). The mean optical density (OD) over the experimental period was highest under co-metabolic conditions. No significant difference in mean OD was observed between the no-TMP and glucose-free conditions, whereas significantly greater growth was observed under

co-metabolic conditions ($p < 0.05$). No apparent lag phase was observed following exposure to TMP, suggesting that the enriched consortium was able to tolerate TMP without requiring a prolonged adaptation period. Total organic carbon (TOC) removal varied among the experimental conditions (Figure 1B). The consortium achieved 13.5 ± 2.29% TOC removal under the TMP-only condition, compared with 84.11 ± 2.35% under the no-TMP condition and 85.77 ± 1.29% under the co-metabolic condition. These results indicate that overall carbon removal was substantially lower in the absence of an additional readily available carbon source. The high TOC removal observed under the co-metabolic condition also indicates that microbial carbon utilization remained active in the presence of TMP.

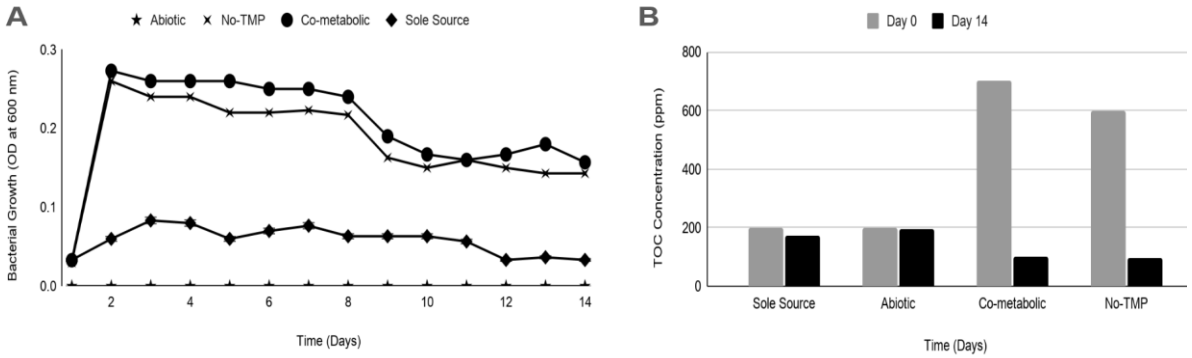


Figure 1. Growth of the bacterial consortium under various conditions (A) and total organic carbon analysis for Day 0 and Day 14 in the different conditions (B).

The bacterial consortium also tolerated a wide range of TMP concentrations (100–500 ppm) (Figure 2). The highest growth was observed at 100 ppm TMP, whereas the lowest growth occurred at 500 ppm. Significant differences in growth were observed among several TMP concentrations. Nevertheless, no apparent lag phase was observed even at the highest TMP concentration. The reduced growth at 500 ppm suggests an inhibitory effect of elevated TMP concentrations on at least a portion of the microbial community. However, continued growth at this concentration indicates that TMP-tolerant or resistant members of the consortium remained metabolically active. A significant difference in OD was observed between cultures exposed to 100 and 500 ppm TMP.

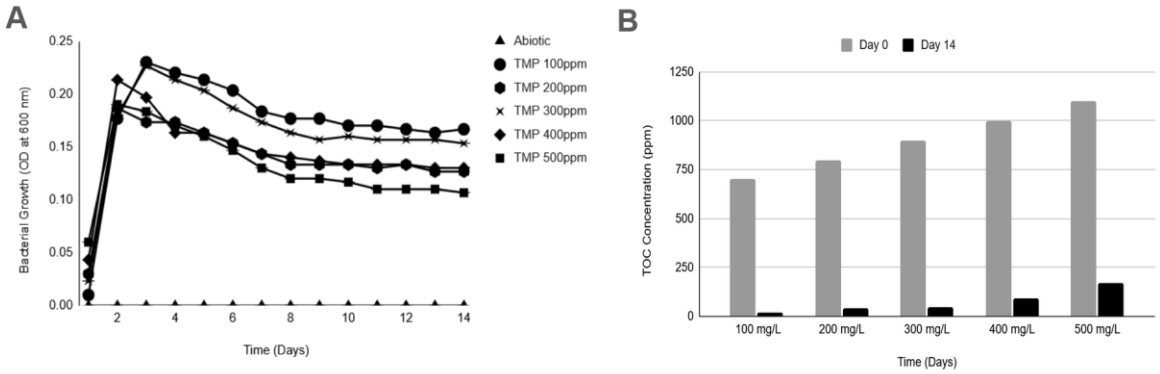


Figure 2. Growth of the consortium in different levels of TMP (A) and total organic carbon degradation of the different concentrations (B).

TOC removal showed a decreasing trend with increasing TMP concentration. The removal efficiencies were 97.16 ± 0.30% at 100 ppm, 95.10 ± 0.82% at 200 ppm, 94.50 ± 0.16% at 300 ppm, 90.70 ± 0.70% at 400 ppm, and 84.50 ± 0.16% at 500 ppm TMP (Figure

2B). All values represent the means of triplicate experiments ± standard deviation. Despite the decreasing trend, no statistically significant differences in TOC removal were detected among the TMP concentrations tested. These findings indicate that substantial carbon removal activity was maintained even at elevated TMP concentrations.

The bacterial consortium enriched from the Thibodaux Sewage Treatment Plant demonstrated a high tolerance to TMP, particularly under co-metabolic conditions with glucose as an auxiliary carbon source. Minimal TMP removal (~2%) occurred under the glucose-free condition, whereas nearly complete removal (~99.78%) was achieved in the presence of glucose. These results indicate that TMP biodegradation by the consortium was strongly enhanced under co-metabolic conditions. This finding is consistent with previous studies demonstrating that co-metabolism can enhance the biodegradation of antibiotics and other organic contaminants by supplying the energy and reducing equivalents required to support microbial growth and transformation reactions [29–31]. For example, ciprofloxacin biodegradation has been reported to occur 16.6–243 times faster under co-metabolic conditions than when the compound was provided without an auxiliary carbon source [32].

Mechanistically, co-metabolism may enhance contaminant transformation by maintaining microbial growth, energy generation, and enzyme activity. In the presence of glucose, active central metabolism can provide energy and reducing equivalents that support enzymatic reactions involved in TMP transformation. Changes in carbon availability may also alter cellular regulatory and stress-response pathways, including cAMP-mediated regulation and responses to oxidative stress [33]. However, these mechanisms were not directly measured in the present study and therefore require further investigation.

The consortium tolerated TMP concentrations of up to 500 ppm, demonstrating substantial resistance to elevated TMP exposure [34,35]. Although growth and TOC removal tended to decrease as TMP concentrations increased, considerable microbial activity was maintained at 500 ppm. Similar effects of auxiliary carbon sources have been observed in co-metabolic systems used to degrade other toxic organic compounds, including phenol [36].

3.2. Metabolic Pathway and Degradation.

High-performance liquid chromatography (HPLC) analysis showed that trimethoprim eluted at 4.53 minutes. No change in TMP concentration was observed in the abiotic controls, confirming biologically mediated degradation. The consortium exhibited minimal TMP removal under TMP as the sole source of carbon conditions (~2%), whereas 98.17% removal was observed under co-metabolic conditions (Figure 3). Complete degradation reached 99.78% after 19 days. Kinetic modeling indicated that TMP degradation was best described by a zero-order model (R^2 = 0.964), compared with the first-order (R^2 = 0.832) and second-order (R^2 = 0.560) models. The better fit of the zero-order model suggests that, under the experimental conditions tested, TMP removal occurred at an approximately constant rate over the evaluated concentration range. Nevertheless, the fit of the first-order model indicates that substrate concentration and co-metabolic interactions may also have influenced the degradation kinetics.

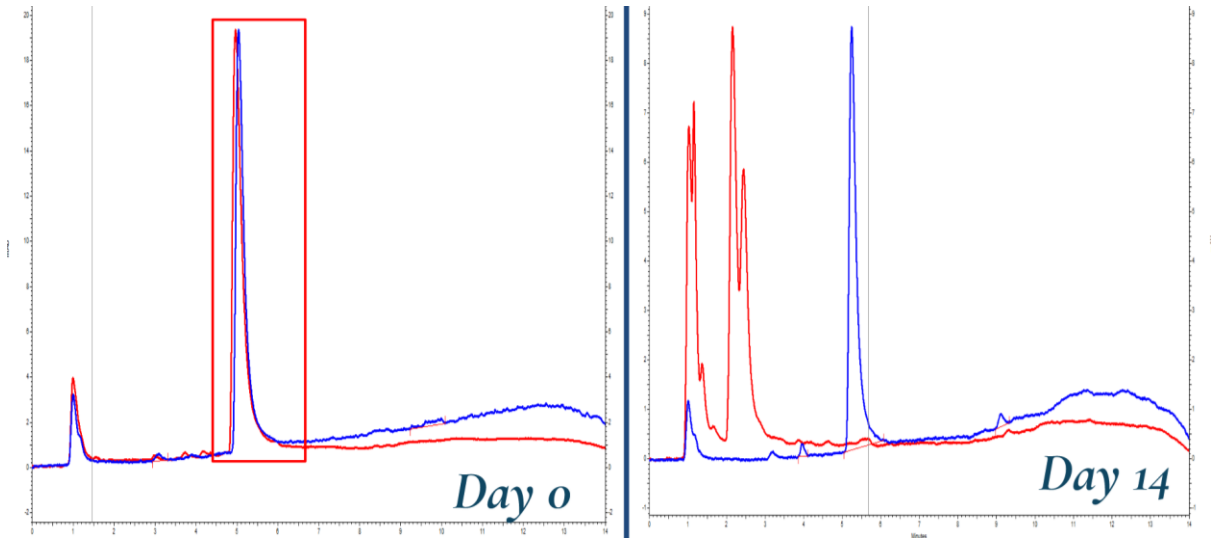


Figure 3. Red Line Showcases the Co-Metabolic Condition With the Consortium and The Blue Line Showcases TMP as the Sole Source of Carbon Conditon With Consortium.

Liquid chromatography–mass spectrometry (LC–MS) analysis confirmed the formation of transformation products, with three major peaks detected. A prominent metabolite with a retention time of 1.57 min and an *m/z* value of 277.1 was assigned to 4-desmethyl trimethoprim. The detection of 4-desmethyl trimethoprim suggests that demethylation was an important initial transformation pathway (Figure 4), consistent with previous reports [19]. The limited detection of additional intermediates may indicate subsequent transformation into other products that were not detected using the analytical approach employed in this study. Although downstream anaerobic processes may contribute to further transformation [37], specific involvement of acidogenesis or acetogenesis cannot be established from the present data.

.

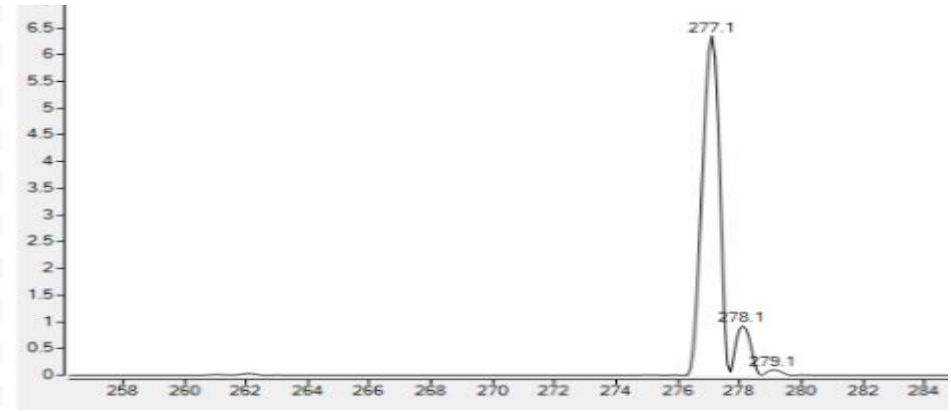


Figure 4. Mass Spectrometry of Day 14 Metabolites in Co-Metabolic Conditions.

3.3. Pure Culture Analysis.

A pure bacterial isolate was obtained to determine whether TMP biodegradation could be attributed to an individual organism or required the activity of the microbial consortium. Under co-metabolic conditions, the isolate exhibited greater growth than the consortium and reached a maximum OD of 0.483. Despite its robust growth, chemical analysis showed no significant TMP degradation by the pure culture. In contrast, substantial TMP degradation was observed with the microbial consortium. These findings suggest that TMP biodegradation under the conditions investigated was dependent on interactions within the microbial consortium rather

than on the activity of the isolated strain alone. The isolate was identified as belonging to the genus *Bacillus*. Previous studies have investigated the enrichment of antibiotic resistance genes in constructed bioelectrochemical systems, river sediments, and activated sludge [38,39]. TMP transformation under sulfate-reducing anaerobic conditions has also been investigated [39]. Although previous studies have reported enrichment of genes belonging to the *dfr* family, the microbial communities and experimental conditions differed from those examined in the present study. Here, a TMP-degrading microbial consortium was enriched directly from anaerobic digester sludge obtained from a local wastewater treatment plant and evaluated under co-metabolic conditions [40]. This approach provides insight into the degradation potential of wastewater-derived microbial communities exposed to TMP.

3.4. Genetic Analysis.

PCR-based genetic analysis confirmed the presence of antibiotic resistance genes in both theconsortium and the pure culture (Figure 5). Both samples contained 16S rRNA bands (~200bp) and dfrA12 gene (~155bp). The consortium uniquely possessed the dfrA5 gene (~351bp), which may contribute to enhanced potential degradation capacity relative to pure culture.

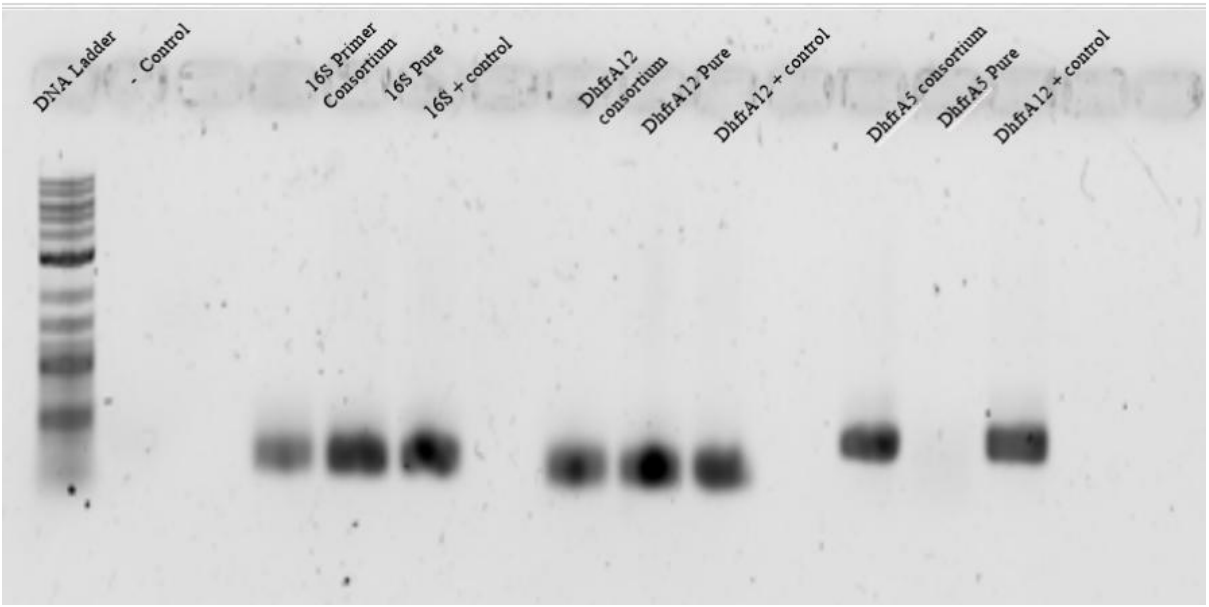


Figure 5. Genetic Analysis of the Consortium and Pure Culture Visualized on PAGE Gel.

The importance of community structure was highlighted in the pure culture analysis. Although the isolated Bacillus strain exhibited robust growth in TMP, it did not significantly degrade the compound. This result indicates that TMP biodegradation can be a consortium-dependent process, likely requiring metabolic interaction among multiple species. Such interactions may involve division of labor, cross-feeding, or complementary enzymatic pathways that are not present in an individual isolate [34]. Genetic analysis further supports this conclusion. Both the consortium and isolate possessed the dfrA12 gene, conferring resistance to TMP. However, the consortium uniquely contained the dfrA5 gene, which may contribute to enhanced tolerance and sustained metabolic activity in the presence of TMP. The presence of multiple resistance genes may allow the consortium to maintain growth while simultaneously supporting degradation processes, whereas individual organisms may remain

resistant but metabolically limited [41]. Research will need to be further expanded on by building a further analysis of the metabolic pathway and also identifying the full bacterial biodiversity in the consortium.

4. Conclusions

Overall, this study demonstrates one of the highest reported levels of TMP degradation under controlled conditions, achieving 99.78% removal within 19 days without extended acclimation. These findings highlight the potential of wastewater-derived microbial consortia as effective agents for microbial removal of TMP. Future work should focus on characterizing downstream metabolic pathways, evaluating degradation under varying redox conditions, and assessing performance at higher TMP concentrations. Once fully characterized, this system has strong potential for integration into wastewater treatment strategies, particularly in coastal regions where improved antibiotic removal would directly reduce pharmaceutical loading into aquatic ecosystems.

Acknowledgments

The present study was funded by the Coypu Foundation.

Author Contribution

TN - Conceptualization, methodology, software, validation, analysis, and writing original draft. BB – Reviewing and editing. DW – Analysis, visualization, HR – Project administration, analysis, writing, validation, reviewing and editing. RB – Conceptualization, validation, project administration, supervision, validation, funding acquisition, and reviewing and editing. AS – Conceptualization, validation, Reviewing and editing. All authors have read and agreed to the published version of the manuscript.

Competing Interest

The authors declare no conflict of interest.

References

[1] Mulchandani, R.; et al. (2025). Global trends in inappropriate use of antibiotics, 2000–2021: scoping review and prevalence estimates. *BMJ Public Health*, *3*, e002411. https://doi.org/10.1136/bmjph-2024-002411.

[2] Klein, E.Y.; et al. (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, E3463–E3470. https://doi.org/10.1073/pnas.1717295115.

[3] Baquero, F.; Lanza, V.F.; Cantón, R.; Coque, T.M. (2015). Public health evolutionary biology of antimicrobial resistance: priorities for intervention. *Evolutionary Applications*, *8*, 223–239. https://doi.org/10.1111/eva.12235.

[4] Gullberg, E.; et al. (2011). Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathogens*, *7*, e1002158. https://doi.org/10.1371/journal.ppat.1002158.

[5] Tao, S.; Chen, H.; Li, N.; Wang, T.; Liang, W. (2022). The Spread of Antibiotic Resistance Genes In Vivo Model. *Canadian Journal of Infectious Diseases and Medical Microbiology*, *2022*, 3348695. https://doi.org/10.1155/2022/3348695.

[6] Kemnic, T.R.; Coleman, M. (2026). Trimethoprim Sulfamethoxazole. In StatPearls; StatPearls Publishing: Treasure Island, FL, USA. (accessed on 11 May 2026) Available online: http://www.ncbi.nlm.nih.gov/books/NBK513232/.

[7] Outpatient Antibiotic Prescriptions — United States, 2017 | Antibiotic Use | CDC. (accessed on 11 May 2026) Available online: https://archive.cdc.gov/www_cdc_gov/antibiotic-use/data/report-2017.html.

[8] Gleckman, R.; Blagg, N.; Joubert, D.W. (1981). Trimethoprim: Mechanisms of Action, Antimicrobial Activity, Bacterial Resistance, Pharmacokinetics, Adverse Reactions, and Therapeutic Indications. *Pharmacotherapy*, *1*, 14–19. https://doi.org/10.1002/j.1875-9114.1981.tb03548.x.

[9] Liu, C.T.; Hanoian, P.; French, J.B.; Pringle, T.H.; Hammes-Schiffer, S.; Benkovic, S.J. (2013). Functional significance of evolving protein sequence in dihydrofolate reductase from bacteria to humans. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 10159–10164. https://doi.org/10.1073/pnas.1307130110.

[10] Toulouse, J.L.; Edens, T.J.; Alejaldre, L.; Manges, A.R.; Pelletier, J.N. (2017). Integron-Associated DfrB4, a Previously Uncharacterized Member of the Trimethoprim-Resistant Dihydrofolate Reductase B Family, Is a Clinically Identified Emergent Source of Antibiotic Resistance. *Antimicrobial Agents and Chemotherapy*, *61*, e02665-16. https://doi.org/10.1128/AAC.02665-16.

[11] Grape, M.; Farra, A.; Kronvall, G.; Sundström, L. (2005). Integrons and gene cassettes in clinical isolates of co-trimoxazole-resistant Gram-negative bacteria. *Clinical Microbiology and Infection*, *11*, 185–192. https://doi.org/10.1111/j.1469-0691.2004.01059.x.

[12] Sánchez-Osuna, M.; Cortés, P.; Llagostera, M.; Barbé, J.; Erill, I. (2020). Exploration into the origins and mobilization of di-hydrofolate reductase genes and the emergence of clinical resistance to trimethoprim. *Microbial Genomics*, *6*, mgen000440. https://doi.org/10.1099/mgen.0.000440.

[13] Toulouse, J.L.; et al. (2020). Dual-Target Inhibitors of the Folate Pathway Inhibit Intrinsically Trimethoprim-Resistant DfrB Dihydrofolate Reductases. *ACS Medicinal Chemistry Letters*, *11*, 2261–2267. https://doi.org/10.1021/acsmedchemlett.0c00393.

[14] Jewell, K.S.; Castronovo, S.; Wick, A.; Falås, P.; Joss, A.; Ternes, T.A. (2016). New insights into the transformation of trimethoprim during biological wastewater treatment. *Water Research*, *88*, 550–557. https://doi.org/10.1016/j.watres.2015.10.026.

[15] Luo, X.; Zheng, Z.; Greaves, J.; Cooper, W.J.; Song, W. (2012). Trimethoprim: Kinetic and mechanistic considerations in photochemical environmental fate and AOP treatment. *Water Research*, *46*, 1327–1336. https://doi.org/10.1016/j.watres.2011.12.052.

[16] Joss, A.; et al. (2006). Biological degradation of pharmaceuticals in municipal wastewater treatment: Proposing a classification scheme. *Water Research*, *40*, 1686–1696. https://doi.org/10.1016/j.watres.2006.02.014.

[17] Guo, J.; et al. (2017). Unraveling microbial structure and diversity of activated sludge in a full-scale simultaneous nitrogen and phosphorus removal plant using metagenomic sequencing. *Enzyme and Microbial Technology*, *102*, 16–25. https://doi.org/10.1016/j.enzmictec.2017.03.009.

[18] Mayhew, M.; Stephenson, T. (1997). Low Biomass Yield Activated Sludge: A Review. *Environmental Technology*, *18*, 883–892. https://doi.org/10.1080/09593331808616607.

[19] Igoni, A.H.; Abowei, M.F.N.; Ayotamuno, M.J.; Eze, C.L. (2008). Effect of Total Solids Concentration of Municipal Solid Waste on the Biogas Produced in an Anaerobic Continuous Digester. *Agricultural Engineering International: CIGR Journal*. (accessed on 11 May 2026) Available online: https://cigrjournal.org/index.php/Ejounral/article/view/1288.

[20] Matheri, A.N.; Nabadda, E.; Mohamed, B. (2023). Sustainable and circularity in the decentralized hybrid solar-bioenergy system. *Environment, Development and Sustainability*, *26*, 16987–17011. https://doi.org/10.1007/s10668-023-03322-w.

[21] Tang, M.; et al. (2020). Enhanced hydrolysis of fermentative antibiotics in production wastewater: Hydrolysis potential prediction and engineering application. *Chemical Engineering Journal*, *391*, 123626. https://doi.org/10.1016/j.cej.2019.123626.

[22] Naquin, A.; Shrestha, A.; Sherpa, M.; Nathaniel, R.; Boopathy, R. (2015). Presence of antibiotic resistance genes in a sewage treatment plant in Thibodaux, Louisiana, USA. *Bioresource Technology*, *188*, 79–83. https://doi.org/10.1016/j.biortech.2015.01.052.

[23] Arceneaux, E.; Nathaniel, K.; Pinel, C.; Cortez, T.; Boopathy, R. (2025). Performance of a Constructed Wetland in Reducing Nutrients, Fecal Coliform Bacteria, and Antibiotic Resistant Bacteria. *Environmental Quality Management*, *35*, e70117. https://doi.org/10.1002/tqem.70117.

[24] Styszko, K.; et al. (2025). Tracking nonregulated micropollutants in sewage sludge: Antimicrobials, OH-PAHs, and microplastics — Environmental risks, fertilizer implications and energy considerations. *Energy Reports*, *13*, 4756–4768. https://doi.org/10.1016/j.egyr.2025.04.013.

[25] Horvath, R.S. (1972). Microbial co-metabolism and the degradation of organic compounds in nature. *Bacteriological Reviews*, *36*, 146–155. https://doi.org/10.1128/br.36.2.146-155.1972.

[26] Chi, Z.; et al. (2023). Co-metabolic biodegradation of chlorinated ethene in an oxygen- and ethane-based membrane biofilm reactor. *Science of the Total Environment*, *905*, 167323. https://doi.org/10.1016/j.scitotenv.2023.167323.

[27] Fischer, K.; Majewsky, M. (2014). Cometabolic degradation of organic wastewater micropollutants by activated sludge and sludge-inherent microorganisms. *Applied Microbiology and Biotechnology*, *98*, 6583–6597. https://doi.org/10.1007/s00253-014-5826-0.

[28] Feng, N.-X.; et al. (2019). Co-metabolic degradation of the antibiotic ciprofloxacin by the enriched bacterial consortium XG and its bacterial community composition. *Science of the Total Environment*, *665*, 41–51. https://doi.org/10.1016/j.scitotenv.2019.01.322.

[29] Jiang, M.; et al. (2023). Ampicillin-controlled glucose metabolism manipulates the transition from tolerance to resistance in bacteria. *Science Advances*, *9*, eade8582. https://doi.org/10.1126/sciadv.ade8582.

[30] De Araujo, C.A.V.; et al. (2017). Simultaneous Removal of the Antimicrobial Activity and Toxicity of Sulfamethoxazole and Trimethoprim by White Rot Fungi. *Water, Air, & Soil Pollution*, *228*, 341. https://doi.org/10.1007/s11270-017-3525-z.

[31] Rodrigues, D.A.D.S.; da Cunha, C.C.R.F.; Pereira, A.R.; Espírito Santo, D.R.D.; Silva, S.Q.; Starling, M.C.V.M.; Santiago, A.D.F.; Afonso, R.J.C.F. (2025). Biodegradation of trimethoprim and sulfamethoxazole in secondary effluent by microalgae-bacteria consortium. *International Journal of Hygiene and Environmental Health*, *264*, 114517. https://doi.org/10.1016/j.ijheh.2024.114517.

[32] Tay, S.T.-L. (2006). Removal of phenol from wastewater by microbial granules. In Waste Management Series, Vol. 6; Elsevier: Amsterdam, The Netherlands; pp. 191–IV. https://doi.org/10.1016/S0713-2743(06)80111-X.

[33] Carneiro, R.B.; Sabatini, C.A.; Titato, G.M.; Ferreira, T.C.R.; Zaiat, M.; Santos-Neto, Á.J. (2022). New biotransformation pathways from sulfamethoxazole and ciprofloxacin removal in sewage treatment along the spatial profile of an anaerobic fixed bed bioreactor. *Bioresource Technology Reports*, *17*, 100944. https://doi.org/10.1016/j.biteb.2022.100944.

[34] Xu, T.; et al. (2025). Prediction of hydrolysis pathways and kinetics of sulfamethoxazole: A machine-learning-based molecular dynamics and experimental study. *Journal of Hazardous Materials*, *494*, 138429. https://doi.org/10.1016/j.jhazmat.2025.138429.

[35] Bergan, T.; Carlsen, I.B. (1985). Effect of antibiotics eliminated by first order kinetics. *Journal of Antimicrobial Chemotherapy*, *15*, 147–152. https://doi.org/10.1093/jac/15.suppl_A.147.

[36] Tsoi, R.; Wu, F.; Zhang, C.; Bewick, S.; Karig, D.; You, L. (2018). Metabolic division of labor in microbial systems. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, 2526–2531. https://doi.org/10.1073/pnas.1716888115.

[37] Saati-Santamaría, Z.; Navarro-Gómez, P.; Martínez-Mancebo, J.A.; Juárez-Mugarza, M.; Flores, A.; Canosa, I. (2025). Genetic and species rearrangements in microbial consortia impact biodegradation potential. *The ISME Journal*, *19*, wraf014. https://doi.org/10.1093/ismejo/wraf014.

[38] Wang, X.; et al. (2025). Efficient anaerobic biodegradation of trimethoprim driven by electrogenic respiration: Optimizing bioelectro-characterization, elucidating biodegradation mechanism and fate of antibiotic resistance genes systematically. *Journal of Hazardous Materials*, *492*, 138070. https://doi.org/10.1016/j.jhazmat.2025.138070.

[39] Liang, B.; Kong, D.; Qi, M.; et al. (2019). Anaerobic biodegradation of trimethoprim with sulfate as an electron acceptor. *Frontiers of Environmental Science & Engineering*, *13*, 84. https://doi.org/10.1007/s11783-019-1168-6.

[40] Ndlela, L.L.; Schroeder, P.; Genthe, B.; Cruzeiro, C. (2023). Removal of Antibiotics Using an Algae-Algae Consortium (*Chlorella protothecoides* and *Chlorella vulgaris*). *Toxics*, *11*, 588. https://doi.org/10.3390/toxics11070588

[41] Zhang, T.; Zhang, H. (2022). Microbial Consortia Are Needed to Degrade Soil Pollutants. *Microorganisms*, *10*, 261. https://doi.org/10.3390/microorganisms10020261.



© 2026 by the authors. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).