Biodegradation of Nicotine by Microorganisms:

Pathways, Environmental Fate, and Bioremediation Potential

Abstract

Nicotine, a chemical compound derived from tobacco, persists in the environment and poses risks to ecosystems and human health. This paper explores microbial degradation mechanisms, covering the primary microorganisms involved, the biochemical pathways employed, and challenges associated with practical application. Laboratory studies demonstrate that microorganisms can efficiently degrade nicotine through specific catabolic pathways, but environmental factors such as soil pH and oxygen availability often inhibit the process under natural conditions. A key finding is that microbial consortia can effectively manage the accumulation of intermediate metabolites, thereby improving remediation outcomes for tobacco waste. Building on last year's topic "Navigating Pharmaceutical Contamination: The Role of Constructed Wetlands in Mitigating Emerging Aquatic Pollutants" and including microbial degradation capabilities and constructed engineering approaches, this paper identifies strategies to enhance the effectiveness and sustainability of microbial bioremediation systems.

Introduction

Nicotine is a natural alkaloid found in tobacco plants, with the molecular formula $C_{10}H_{14}N_2$. It exhibits basic properties (pKa 8.02), moderate hydrophobicity (log K_ow 1.17), and high water solubility, which facilitates its rapid transport through soil and aquatic systems (PubChem, 2023). Environmental contamination occurs primarily through tobacco manufacturing waste, discarded cigarette filters, agricultural runoff, and electronic cigarette liquids (World Health Organization, 2023). A single cigarette butt can release nearly 1 mg of nicotine into water within 24 hours, resulting in widespread contamination (Green et al., 2014).

In natural environments, nicotine disrupts microbial community structure in soil and aquatic ecosystems, exhibits neurotoxic effects on fauna, and undergoes transformation into tobacco-specific nitrosamines (TSNAs), which are carcinogenic. Human health impacts include neurological effects and addiction, as well as indirect exposure risks through contaminated water sources. Cotinine, a stable metabolite of nicotine, serves as a biomarker for tobacco pollution in rivers and wastewater treatment systems. This compound forms through partial degradation of nicotine, either through biological metabolism or photochemical reactions, and persists longer in the environment. In coastal waters and sewage systems, cotinine concentrations indicate ongoing tobacco contamination (Buerge et al., 2008).

Microorganisms function as biological agents for the degradation of persistent pollutants such as nicotine, utilizing it as a carbon and nitrogen source for energy and growth under aerobic

conditions. This paper examines the gap between controlled laboratory degradation studies and field-scale applications, where variable environmental conditions often limit complete mineralization. The review synthesizes current understanding of degradation pathways, factors influencing nicotine fate in the environment, and strategies for developing practical bioremediation systems, with particular emphasis on the advantages of microbial consortia over monocultures.

Microbial Diversity and Isolation

A diverse array of bacteria and fungi capable of nicotine degradation has been identified, predominantly isolated from tobacco-contaminated environments. Common bacterial genera include Pseudomonas (e.g., P. putida), Arthrobacter (e.g., A. nicotinovorans), Agrobacterium, Bacillus, Ochrobactrum, Rhodococcus, Acinetobacter, Sphingomonas, and Ensifer (Liu et al., 2015; Tang et al., 2013). These microorganisms are isolated from diverse sources including rhizosphere soil near tobacco fields, waste disposal sites, contaminated groundwater, and water exposed to cigarette waste. Isolation procedures employ selective media containing nicotine as the sole carbon source, with cultivation under aerobic conditions at temperatures ranging from 20–37°C and pH of 6.4–7.5. Enrichment cultures undergo serial transfer to select for organisms with high degradation capacity. Initial screening may utilize tobacco waste on selective agar plates, favoring rapid-growing or spore-forming species (Xu et al., 2007).

The genetic basis for nicotine degradation often involves catabolic gene clusters located on plasmids or chromosomes, indicating horizontal gene transfer among soil bacteria (Mihasan & Brandsch, 2013). This genetic diversity is functionally significant because individual species rarely possess complete mineralization capability. In natural environments, microbial consortia facilitate sequential degradation, with partially metabolized intermediates transferred between species, thereby preventing accumulation of toxic compounds. Isolation studies demonstrate that nicotine-degrading microorganisms are widespread in contaminated environments, though their activity depends on appropriate environmental conditions.

Biochemical Pathways of Nicotine Catabolism

Microorganisms degrade nicotine through two primary catabolic routes, both ultimately resulting in complete mineralization to CO_2 and ammonia.

The pyrrolidine pathway, commonly observed in Pseudomonas species, proceeds through sequential transformations: nicotine is oxidized to N-methylmyosmine, then to pseudooxynicotine, 3-succinoylpyridine, and finally to succinate and fumarate, which enter central metabolic pathways. Key enzymes include nicotine dehydrogenase (Ndh), ketone reductase, and 6-hydroxy-3-succinoylpyridine hydroxylase (Hsp) (Tang et al., 2008; Tang et al., 2013).

The pyridine pathway, characteristic of Arthrobacter species, follows an alternative route: nicotine is hydroxylated to 6-hydroxy-nicotine, then converted to 6-hydroxy-N-methylmyosmine, 2,5-dihydroxypyridine, maleamate, and fumarate. This pathway utilizes enzymes including 6-hydroxy-L-nicotine oxidase (Hno) and 6-hydroxy-D-nicotine oxidase (Hdo) (Brandsch, 2006; Baitsch et al., 2001).

Comparative analysis reveals distinct characteristics: the pyrrolidine pathway exhibits faster kinetics but is sensitive to pH fluctuations, while the pyridine pathway demonstrates more complete degradation but proceeds at a slower rate. Intermediate metabolites such as 6-hydroxynicotine can accumulate and inhibit further degradation in pure cultures. However, microbial consortia overcome this limitation through metabolic cooperation, wherein one species' metabolic products serve as substrates for another. This sequential metabolism enables complete degradation, releasing energy for microbial growth while converting a pollutant into non-toxic end products.

Microbial Metabolism and Energy Requirements for Nicotine Degradation

Understanding how microorganisms derive energy and nutrients from nicotine requires examining the fundamental metabolic requirements that govern microbial degradation processes. Nicotine-degrading microorganisms must satisfy five essential metabolic needs: an energy source, a carbon source, a terminal electron acceptor, additional nutrients, and appropriate environmental conditions.

Energy Source and Carbon Source

Nicotine-degrading bacteria function as chemoorganotrophs, obtaining energy by breaking chemical bonds in organic compounds—in this case, nicotine itself. The degradation of nicotine releases electrons from the reduced carbon and nitrogen atoms in its structure ($C_{10}H_{14}N_2$), which are then captured through oxidation reactions catalyzed by specific enzymes. In the pyrrolidine pathway, nicotine dehydrogenase (Ndh) catalyzes the initial oxidation of nicotine to N-methylmyosmine, removing electrons that enter the electron transport chain for ATP generation (Tang et al., 2013). Similarly, in the pyridine pathway, 6-hydroxy-L-nicotine oxidase (Hno) performs oxidation reactions that release energy-rich electrons (Brandsch, 2006; Baitsch et al., 2001).

These same microorganisms function as heterotrophs, utilizing nicotine as their carbon source. The carbon atoms from nicotine's pyridine and pyrrolidine rings are progressively broken down through enzymatic pathways, ultimately yielding simple compounds such as succinate and fumarate (Tang et al., 2013). These intermediates feed directly into central metabolic pathways where they are further oxidized to CO_2 while generating additional energy. This dual role—nicotine serving as both energy source and carbon source—makes it an ideal growth substrate for adapted microorganisms. Studies of Pseudomonas putida strain S16 demonstrated

that cells grown on nicotine as the sole carbon and nitrogen source exhibited robust growth, confirming complete integration of nicotine-derived carbon into cellular biomass (Xu et al., 2007).

The nitrogen content of nicotine (two nitrogen atoms per molecule) provides an additional metabolic advantage. As microorganisms cleave the pyrrolidine ring and oxidize the pyridine ring, nitrogen is released as ammonia (NH_3), which can be directly assimilated into amino acids and nucleotides (Brandsch, 2006; Moreau et al., 2019). This nitrogen liberation represents a significant benefit in nitrogen-limited environments, where nicotine degradation simultaneously addresses pollution while supplying essential nutrients to the microbial community (Liu et al., 2015).

Terminal Electron Acceptor

The efficiency of nicotine degradation depends critically on the availability of terminal electron acceptors to receive the electrons extracted during oxidation. Most characterized nicotine-degrading bacteria employ aerobic respiration, using molecular oxygen (O_2) as the terminal electron acceptor (Xu et al., 2007; Liu et al., 2015). Oxygen's high reduction potential makes it the most efficient terminal electron acceptor for energy generation. In the pyrrolidine pathway, multiple hydroxylase and oxidase enzymes require molecular oxygen as a co-substrate for hydroxylation reactions, making oxygen essential not only as a terminal electron acceptor but also as a direct participant in degradation chemistry (Tang et al., 2008).

However, oxygen limitation represents a major constraint in natural environments. In waterlogged soils, sediments, or deeper soil layers, oxygen concentrations drop significantly. Current research indicates that nicotine degradation under strictly anaerobic conditions is significantly slower and often incomplete, as the key oxidative enzymes (Ndh, Hno, Hdo) in characterized pathways require oxygen (Tang et al., 2013; Brandsch, 2006). This oxygen dependence explains why nicotine persists longer in anaerobic environments such as waterlogged tobacco fields or deep sediments compared to well-aerated surface soils (Karwautz & Lueders, 2020).

Additional Nutrient Requirements

Beyond carbon, nitrogen, and energy, nicotine-degrading microorganisms require phosphorus for nucleic acid and membrane synthesis, sulfur for amino acids like cysteine and methionine, and various trace elements. Iron is particularly important as a cofactor for many oxidase and hydroxylase enzymes involved in nicotine degradation. For example, the hydroxylase enzymes in both the pyrrolidine and pyridine pathways contain iron-sulfur clusters that facilitate electron transfer during oxidation reactions (Tang et al., 2008; Baitsch et al., 2001).

In bioremediation applications, nutrient availability can influence degradation efficiency. Research on soil microbial communities has shown that nutrient amendments can alter microbial activity and community composition (Geisseler & Scow, 2014). When nicotine is the sole nitrogen source, its degradation releases ammonia that can support not only the degrader population but also the broader microbial community, creating a self-sustaining system (Xu et al., 2007).

Environmental Conditions

Supporting environmental conditions govern enzyme activity and microbial physiology. Temperature affects reaction kinetics, with optimal nicotine degradation typically occurring between 25-30°C for mesophilic species like Pseudomonas putida and Arthrobacter nicotinovorans (Liu et al., 2015; Xu et al., 2007). pH exerts strong effects through multiple mechanisms: it influences nicotine speciation (protonation state), enzyme activity, and membrane transport (PubChem, 2023). pH 7-8 is optimal for most bacterial degraders, though specific enzymes may have different pH optima (Xu et al., 2007).

Salinity can also affect degradation efficiency, with different species exhibiting varying salt tolerances (Wichern et al., 2020). Toxic compounds—including heavy metals or high concentrations of nicotine itself—can inhibit degradation. These environmental constraints explain why laboratory degradation rates often exceed field performance, where conditions fluctuate beyond optimal ranges (Liu et al., 2015).

Metabolic Integration and Consortial Advantages

The metabolic requirements outlined above reveal why microbial consortia often outperform pure cultures in natural systems (Liu et al., 2015). Different species within a consortium may have complementary metabolic capabilities, and syntrophic relationships can develop where one organism's waste products serve as substrates for another, enabling complete mineralization even when no single species possesses all necessary enzymes (Saini et al., 2021; Sharma et al., 2017). Additionally, some consortium members may provide supporting functions—such as removing toxic intermediates or creating favorable microenvironments through biofilm formation. This metabolic cooperation explains the success of mixed cultures in both natural attenuation and engineered bioremediation systems (Liu et al., 2015).

Fungal Degradation Mechanisms

While bacteria dominate nicotine degradation research, fungi provide complementary degradative capacity through fundamentally different biochemical strategies. Unlike bacteria, which rely on specific catabolic enzymes within metabolic pathways, fungi utilize extracellular oxidative enzymes—including lignin peroxidase, manganese peroxidase, and laccase—that

exhibit low substrate specificity and can oxidize aromatic and heterocyclic compounds through non-specific oxidation reactions.

Aspergillus species, particularly A. oryzae, have demonstrated notable nicotine degradation capacity. Research by Meng et al. (2010) identified a novel pathway in A. oryzae strain 112822, isolated from tobacco leaves, which proceeds through hydroxylation reactions, producing intermediates including nicotine-N-oxide and cotinine before achieving mineralization. The fungus degraded nicotine as a sole carbon and nitrogen source under aerobic conditions at neutral pH and moderate temperatures (28-30°C). Fungal degradation offers practical advantages in bioremediation: fungi tolerate wider environmental ranges including lower pH (4-6) and reduced oxygen availability, making them suitable for conditions where bacterial activity is limited. Their hyphal growth enables physical penetration of soil matrices, accessing sequestered nicotine unavailable to bacteria.

The integration of fungal and bacterial degraders in mixed consortia represents a particularly promising approach. In such systems, bacteria rapidly metabolize readily available nicotine through specific catabolic pathways, while fungi address accumulated intermediates and recalcitrant compounds through oxidative enzymes. This complementary functionality demonstrates that effective bioremediation can leverage the distinct metabolic capabilities of both bacteria and fungi.

Physicochemical Factors and Microbial Efficiency

Environmental parameters significantly influence both nicotine behavior and microbial degradation efficiency. Nicotine's pKa of 8.02 indicates weak base character, with increased protonation in acidic conditions resulting in enhanced sorption to soil particles and reduced mobility. The log $K_o w$ of 1.17 reflects moderate hydrophobicity, sufficient to enable binding to organic matter while maintaining substantial water solubility for transport through saturated soils. These properties directly affect bioavailability; in neutral to slightly alkaline soils (pH 7-8), nicotine exists predominantly in the unprotonated form, exhibiting greater mobility and accessibility to microorganisms.

Optimization of degradation conditions requires careful consideration of multiple parameters. Maintaining soil pH between 7-8 enhances pyrrolidine pathway activity in Pseudomonas species, while adequate oxygen availability is essential for aerobic metabolism. Nutrient supplementation, particularly carbon and nitrogen sources, supports microbial growth without substrate inhibition. Temperature optimization (25-30°C for most degraders) and moisture control maximize enzyme activity. In practical applications, engineered systems incorporating these parameters can significantly enhance bioremediation efficiency at contaminated sites.

Constructed Wetlands for Nicotine Remediation

Constructed wetlands (CWs) represent a low-cost approach for harnessing microbial degradation capacity to remove nicotine and cotinine from wastewater. These engineered systems consist of shallow basins containing gravel substrate, emergent vegetation such as Phragmites or Typha species, and controlled water flow (Matamoros et al., 2008). They are designed to be able to treat wastewater from tobacco processing facilities or agricultural operations.

At the microscale level, influent nicotine adsorbs to plant roots and substrate particles, reducing transport velocity and concentrating the contaminant in the bioactive zone. The rhizosphere provides aerobic microsites through oxygen release from roots, creating favorable conditions for Pseudomonas and Arthrobacter populations. These organisms colonize root surfaces, forming biofilms that facilitate metabolic cooperation. Initial pyrrolidine pathway degradation by one species produces intermediates that serve as substrates for completion of mineralization by other species. Fungal populations, including white-rot species, contribute enzymatic capacity for degradation of recalcitrant compounds such as cotinine.

Hydraulic retention time (HRT) determines substrate-microbe contact duration, with optimal removal (80-90% based on studies of structurally similar alkaloids) typically achieved at 5-7 days (Auvinen et al., 2017; Wang & Dzakpasu, 2023). While plant uptake contributes to removal, microbial mineralization represents the primary mechanism, converting nicotine to CO_2 and inorganic nutrients that support wetland productivity. Operational challenges include temperature-dependent reduction in microbial activity during cold periods and system overload at high contaminant concentrations.

Conclusion

Microbial degradation of nicotine represents a viable bioremediation approach, with diverse bacterial and fungal species capable of utilizing nicotine as a carbon and nitrogen source through well-characterized catabolic pathways. Laboratory studies demonstrate efficient degradation under controlled conditions, yet field applications face challenges from variable environmental parameters including pH, oxygen availability, and temperature fluctuations. The integration of microbial consortia overcomes limitations of individual species by facilitating sequential metabolism and preventing toxic intermediate accumulation.

Constructed wetlands exemplify practical implementation of microbial degradation principles, combining passive treatment with sustainable design. Future research directions should focus on optimization of environmental parameters for field applications, development of cold-tolerant microbial strains, and integration of bioaugmentation strategies with engineered systems. Enhanced understanding of microbial community dynamics and metabolic cooperation will enable more effective remediation of tobacco-contaminated environments, contributing to both ecosystem protection and human health.

References

Auvinen, H., Gebhardt, W., Linnemann, V., Du Laing, G., & Rousseau, D. P. L. (2017). Laboratory- and full-scale studies on the removal of pharmaceuticals in an aerated constructed wetland: Effects of aeration and hydraulic retention time on the removal efficiency and assessment of the aquatic risk. *Water Science & Technology*, *76*(6), 1457–1465. https://doi.org/10.2166/wst.2017.328

Baitsch, D., Sandu, C., Brandsch, R., & Igloi, G. L. (2001). Gene cluster on pAO1 of Arthrobacter nicotinovorans involved in degradation of the plant alkaloid nicotine: Cloning, purification, and characterization of 2,6-dihydroxypyridine 3-hydroxylase. *Journal of Bacteriology*, *183*(18), 5262–5267. https://doi.org/10.1128/JB.183.18.5262-5267.2001

Brandsch, R. (2006). Microbiology and biochemistry of nicotine degradation. *Applied Microbiology and Biotechnology*, *69*(5), 493–498. https://doi.org/10.1007/s00253-005-0226-0

Buerge, I. J., Kahle, M., Buser, H.-R., Müller, M. D., & Poiger, T. (2008). Nicotine derivatives in wastewater and surface waters: Application as chemical markers for domestic wastewater. *Environmental Science & Technology*, *42*(17), 6354–6360. https://doi.org/10.1021/es800455q

Geisseler, D., & Scow, K. M. (2014). Long-term effects of mineral fertilizers on soil microorganisms – A review. *Soil Biology and Biochemistry*, *75*, 54–63. https://doi.org/10.1016/j.soilbio.2014.03.023

Karwautz, C., & Lueders, T. (2020). Impact of hydraulic well restoration on native bacterial communities in drinking water wells. *Microorganisms*, *8*(8), 1238. https://doi.org/10.3390/microorganisms8081238

LibreTexts. (n.d.). *18.3: Aerobic Respiration*. Biology LibreTexts. https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_(Kaiser)/Unit_7%3A_Microbial_Genetics_and_Microbial_Metabolism/18%3A_Microbial_Metabolism/18.3%3A_Aerobic_Respiration

LibreTexts. (n.d.). *6.1B: Sources of Essential Nutrients*. Biology LibreTexts. https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_(Boundless)/06%3A_Culturing_Microorganisms/6.01%3A_Microbial_Nutrition/6.1B%3A_Sources_of_Essential_Nutrients

Liu, J., Ma, G., Chen, T., Hou, Y., Yang, S., Zhang, K.-Q., & Yang, J. (2015). Nicotine-degrading microorganisms and their potential applications. *Applied Microbiology and Biotechnology*, *99*(9), 3775–3785. https://doi.org/10.1007/s00253-015-6525-1

Matamoros, V., García, J., & Bayona, J. M. (2008). Organic micropollutant removal in a full-scale surface flow constructed wetland fed with secondary effluent. *Water Research*, *42*(3), 653–660. https://doi.org/10.1016/j.watres.2007.08.016

Meng, X. J., Lu, L. L., Gu, G. F., & Xiao, M. (2010). A novel pathway for nicotine degradation by Aspergillus oryzae 112822 isolated from tobacco leaves. *Research in Microbiology*, *161*(7), 626–633. https://doi.org/10.1016/j.resmic.2010.05.017

Mihasan, M., & Brandsch, R. (2013). pAO1 of Arthrobacter nicotinovorans and the spread of catabolic traits by horizontal gene transfer in gram-positive soil bacteria. *Journal of Molecular Evolution*, *77*(1-2), 22–30. https://doi.org/10.1007/s00239-013-9576-x

Moreau, D., Bardgett, R. D., Finlay, R. D., Jones, D. L., & Philippot, L. (2019). A plant perspective on nitrogen cycling in the rhizosphere. *Functional Ecology*, *33*(4), 540–552. https://doi.org/10.1111/1365-2435.13303

Oregon State University. (n.d.). *Chemoorganotrophy*. General Microbiology. https://open.oregonstate.education/generalmicrobiology/chapter/chemoorganotrophy/

PubChem. (2023). Nicotine. National Center for Biotechnology Information. https://pubchem.ncbi.nlm.nih.gov/compound/Nicotine

Saini, R., Kapoor, R., Kumar, R., Siddiqi, T. O., & Kumar, A. (2021). Nicotine waste generated from tobacco industry as a potential source for bioremediation of industrial effluents. *Environmental Science and Pollution Research*, *28*(42), 59003–59014. https://doi.org/10.1007/s11356-021-16297-4

ScienceDirect. (n.d.). *Heterotrophy*. ScienceDirect Topics. https://www.sciencedirect.com/topics/immunology-and-microbiology/heterotrophy

Sharma, B., & Lin, C. Y. (2017). Effects of nicotine on ammonia, particulate matter and microbial characteristics of the biodrying process. *Waste Management*, *63*, 87–97. https://doi.org/10.1016/j.wasman.2016.09.016

Tang, H., Wang, S., Ma, L., Meng, X., Deng, Z., Zhang, D., & Ma, C. (2008). A novel gene, encoding 6-hydroxy-3-succinoylpyridine hydroxylase, involved in nicotine degradation by Pseudomonas putida strain S16. *Applied and Environmental Microbiology*, *74*(5), 1567–1574. https://doi.org/10.1128/AEM.02529-07

Tang, H., Wang, L., Wang, W., Yu, H., Zhang, K., Yao, Y., & Xu, P. (2013). Systematic unraveling of the unsolved pathway of nicotine degradation in Pseudomonas. *PLOS Genetics*, *9*(10), Article e1003923. https://doi.org/10.1371/journal.pgen.1003923

Wang, X. C. C., & Dzakpasu, M. (2023). Removal of pharmaceutical active compounds in wastewater by constructed wetlands: Performance and mechanisms. *Journal of Environmental Management*, *325*(Part A), Article 116478. https://doi.org/10.1016/j.jenvman.2022.116478

Wichern, F., Islam, M. R., Hemkemeyer, M., Watson, C., & Joergensen, R. G. (2020). Organic amendments alleviate salinity effects on soil microorganisms and biochemical properties in a black soil of Northern Germany. *Applied Soil Ecology*, *153*, 103639. https://doi.org/10.1016/j.apsoil.2020.103639

World Health Organization. (2023). Tobacco: Key facts. https://www.who.int/news-room/fact-sheets/detail/tobacco

Xu, P., Han, X., Tang, H., & Wang, S. N. (2007). Characterization of environmentally friendly nicotine degradation by Pseudomonas putida biotype A strain S16. *Microbiology*, *153*(5), 1556–1565. https://doi.org/10.1099/mic.0.2006/005223-0