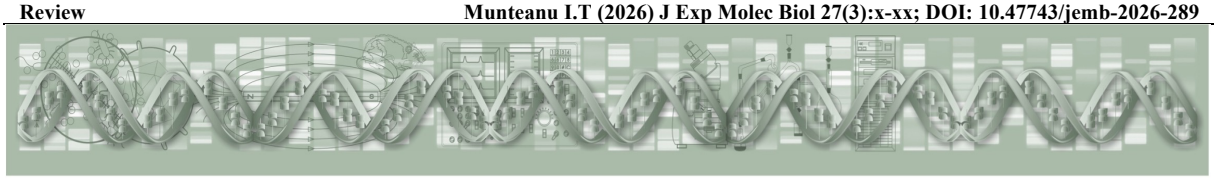

MICROBIAL VALORIZATION OF NICOTINE AND TOBACCO WASTE FOR BIOACTIVE COMPOUNDS RECOVERY: A SYSTEMATIC REVIEW

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Abstract

The tobacco industry produces thousands of tons of toxic waste annually, containing nicotine at concentrations ranging from 3% to 6% (w/w). Traditional waste management approaches entail high costs and negative impacts on the environment and human health. Microbial biotechnology may be used for the simultaneous detoxification of nicotine and recovery of bioactive compounds from tobacco waste (TW). The systematic review is based on the guidelines established by PRISMA 2020 and seeks to find data on bacteria-based approaches to TW valorization, structured around the PICO framework. The literature search was conducted between April and June 2026 across Scopus, Web of Science (WoS), PubMed, and Google Scholar, supplemented by reports from World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO), and Grand View Research. Following duplicate removal and screening of 201 studies, 13 research articles and 7 organizational reports met the inclusion criteria. Of the 13 studies, 7 focused on NDM strains, 4 on nicotine-tolerant non-NDMs, and 2 on co-culture systems. Functional pyridines were the most frequently recovered compounds, with pharmaceutical applications being the predominant target sector. The findings confirm that microbial valorization of TW aligns well with circular economy principles. Nevertheless, some limitations must be acknowledged: the screening process was conducted by a single author, and the considerable heterogeneity of experimental conditions across the included studies precluded a quantitative meta-analysis, making a narrative synthesis the only viable approach. Future research should prioritize scale-up studies and safety validation of the produced compounds.

Introduction

The tobacco industry is one of the largest and most controversial markets in the world. Its global market venue was estimated at 926 billion USD in 2025 (*1*), while according to WHO, annual cost of treatment and productivity loss associated with smoking is higher than 1 trillion USD (*2*). Tobacco kills around 7 million people around the world and 1.1 million people each year in the European Union (EU). People who smoke have 2 - 4 times higher risks of having heart disease and suffering a stroke than nonsmokers (*3*, *4*). However, tobacco consumption in the past ten years has increased by 25% - 30%. The increase in consumption is particularly high among young population. The reason behind such trend is aggressive marketing strategy applied by the industry and introduction of new heated tobacco products (HTPs). In general, HTPs are presented as much healthier alternatives to cigarette smoking. These factors led to an increase in tobacco mortality of 15.4% among men and 4.7% among women in EU (*3*).

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For most people, the harm caused to society by the tobacco industry is primarily associated with health issues. However, the environmental impacts it generates are rarely taken into consideration. The process of farming tobacco is very intensive since it requires high amounts of pesticides and fertilizers that are extremely toxic chemicals that pollute the groundwater (*5*, *6*). The largest tobacco producing nations include China, India, Brazil, the United States, countries across Southeast Asia, and those in sub-Saharan Africa. Worldwide, the production of tobacco plants is estimated to cover about 4.3 million hectares of land, while 200.000 hectares of forest are felled yearly in order to grow and cure tobacco. This results in deforestation at 2% - 4%, which is equivalent to the loss of about 600 million trees per annum (*1*, *5*, *6*). Additionally, 22 billion tons of water are consumed for irrigating crops annually, while the production chain generates around 2 million tons of solid waste each year, including 300.000 tones of nicotine-contaminated residues and 200.000 tones of chemical by-products, making TW particularly difficult to manage and dispose of safely (*5*–*7*).

The annual cost involved in the treatment of TW reach 2.6 billion USD in China, 766 million USD in India, 234 million USD in Germany and over 200 million USD in Brazil (*6*). In response, WHO has called on countries to prioritize tobacco control as part of their commitments to the 2030 Agenda for Sustainable Development Goals (*5*).

Traditional approaches such as disposal through landfills, burning, pyrolysis, gasification, and chemical solvent neutralization not only come at a cost but are also harmful to the environment (*7*). In addition to its impact on health, the large amount of waste generated annually by the tobacco industry also represents an untapped resource that, if used efficiently, could contribute significantly to the adoption of a circular economy principles in this sector.

Recently, an increasing number of studies have been published focusing on research into various environmentally friendly methods for recycling TW. In particular, the use of NDMs represents one of the most promising approaches. NDMs are capable of using nicotine as a sole source of carbon and energy, playing a role in the bioremediation of TW by neutralizing nicotine toxicity. Nicotine degradation involves several metabolic pathways (pyridine, pyrrolidine, and VPP) which yield functional pyridines with potential for use in the production of bioactive compounds applicable in different domains. In addition to NDMs, other bacterial strains capable of tolerating nicotine without degrading it have also shown considerable biotechnological potential for the valorization of TW, contributing to the recovery of commercially relevant compounds.

However, the current scientific literature on this topic is fragmented, and the integration of these techniques has not yet been well systematized. In this context, the main goal of this systematic review is to explore the most significant existing scientific findings regarding the application of bacterial biotechnology in the biological valorization of waste generated by the tobacco industry, with a special focus on the recovery of bioactive compounds that can be used in the pharmaceutical, agricultural, environmental chemistry, and food industries.

Materials and Methods

Search strategy

The systematic literature review was performed according to the PRISMA 2020 guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). The PRISMA 2020 checklist, provided in Appendix 1, was used to guide the reporting and organization of this systematic review. These guidelines were used to define the search strategy, select databases, identify keywords for the search, and establish inclusion and exclusion criteria (*8*, *9*).

The literature research was conducted from April to June 2026 and involved two research areas. The literature related to the scope of the tobacco industry worldwide and its adverse impact on the environment and human health, served as background information for the introduction section, was identified through the WHO, the FAO, and Grand View Research. The relevant

literature on the valorization of nicotine and TW, and recovery of bioactive compounds using bacterial methods was conducted in four international databases: Scopus, WoS, PubMed, and Google Scholar.

The following Boolean operators were used to create various combinations of the chosen keywords within search strings: ′tobacco waste AND circular economy′, ′tobacco waste AND nicotine-degrading microorganisms′, ′nicotine AND microorganisms′, ′tobacco waste OR tobacco residues′, ′nicotine detoxification OR nicotine decontamination′, ′circular economy AND bioactive compounds AND nicotine-degrading microorganisms′, ′tobacco waste AND nicotine degradation′. Search was applied to the title, abstract, and keyword sections of indexed articles whenever possible. In addition, the reference list of the selected studies was screened to identify additional relevant publications. All the obtained documents were collected in a single Excel worksheet (Microsoft Corporation, SUA), and duplicates were manually removed.

Inclusion and exclusion criteria

The systematic review followed the PICO methodology as indicated into the PRISMA 2020 guidelines: ***P****opulation* – nicotine and TW; ***I****ntervention* – biotechnological bacterial techniques, namely NDMs, novel NDMs, nicotine-tolerant non-NDMs, and co-cultures comprising both NDMs and non-NDMs; ***C****omparison* – no specific comparison among the investigated approaches was performed, as the included studies were evaluated individually through a narrative synthesis approach; ***O****utcome* – recovery of bioactive compounds with different industrial applications.

Studies were included if they satisfied the following criteria: i. reports containing data on the global scale of the tobacco industry and the associated human health and environmental issues, ii. original research articles published after 2014 in journals with an impact factor (IF) ≥ 1.5, iii. studies focused on the identification of NDMs or other bacterial approaches for the recovery of valuable bioactive compounds from nicotine and TW, and iv. articles written in English, with the full text available. Studies were excluded if they met any of the following criteria: lack of empirical experimental data, articles focused exclusively on NDMs classification or the description of metabolic pathways without biotechnological relevance, genomic analysis studies, standard decontamination methods (pyrolysis, incineration, and physical-chemical neutralization), decontamination methods that did not focus on the recovery of bioactive compounds, and studies involving fungi in the decontamination of nicotine or TW.

Study selection and data extraction

Each paper was manually reviewed by a single author based on predefined criteria described in the section above. The studies were evaluated with regard to microbial bacteria-based approaches for the valorization of nicotine and TW, with a focus on the biotechnological recovery of valuable bioactive compounds and their potential applications in pharmacy, agriculture, and green chemistry, in line with the principles of the circular economy. The results were organized thematically according to the bacterial approach employed, distinguishing among NDM applications, novel NDM applications, nicotine-tolerant non-NDMs, and co-culture systems. No formal assessment of risk of bias was performed.

AI-assisted tools

Artificial intelligence-assisted tools were used during the preparation and organization of this systematic review. NotebookLM (Google, 2025) was used to synthesize and organize the key ideas from the included studies. DeepL (DeepL GmbH, Germany) was used for the initial translation of the selected content, while Claude Sonnet 4.6 (Anthropic, USA) was used to support subsequent linguistic refinement and manuscript improvement. All content was critically reviewed and validated by the author.

Results

The total number of reports obtained from international organizations and market research companies was 22, while another 225 articles were found in international academic databases. This distribution is as follows: WHO (n = 13), FAO (n = 8), Grand View Research (n = 1), Scopus (n = 41), WoS (n = 72), PubMed (n = 57), and Google Scholar (n = 55). After removing duplicates, a total of 201 documents were screened for relevance, of which 17 were organizational reports and 184 were academic articles from international databases. Following this stage, a total of 6 organizational reports and 81 research articles that did not satisfy the inclusion criteria were excluded. During the secondary screening phase, an additional 4 organizational reports and 90 articles from databases were excluded.

In the final stage, 7 organizational reports and 13 research articles satisfied all inclusion criteria and were included in the qualitative review. The organizational reports were retrieved from the WHO (n = 6) and Grand View Research (n = 1), while the research articles were identified through Scopus (n = 2), PubMed (n = 7), and Web of Science (n = 4). PubMed provided the most specific studies on microbial degradation of nicotine, NDMs characterization, and metabolic pathways. The articles from Scopus included molecular investigations of the subject, as well as of the genome and proteome of NDMs. The largest variety of articles was generated by WoS, these covered various aspects of TW valorization, including the application of the circular bioeconomy using NDMs or different bacterial approach. As a final result, Google Scholar was used as a supplementary source for screening, however no additional eligible studies were identified for inclusion. The general study selection process is illustrated in the PRISMA 2020 flow diagram (Fig. 1), assuring transparency and reproducibility in accordance with systematic review standards.

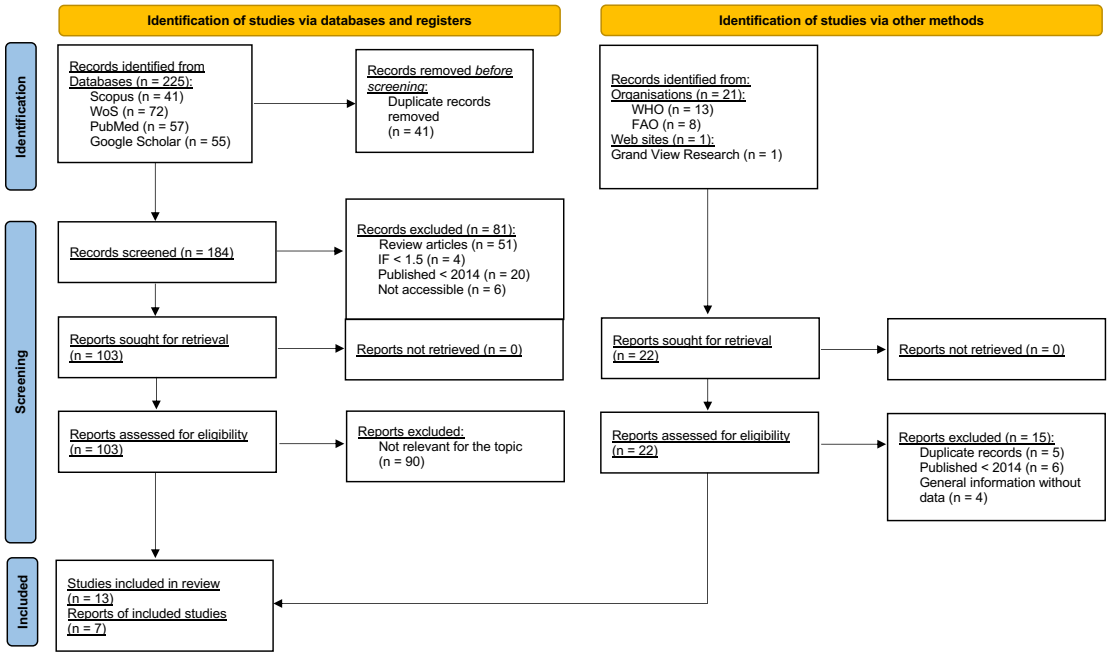

Fig 1. PRISMA 2020 flow diagram for systematic review.

Out of the 13 studies included in this systematic review, 7 have focused on NDMs, 4 investigated nicotine-tolerant non-NDMs, and the other 2 examined co-cultures systems. In terms of compound classes, functional pyridines appear to be the most common one among the reviewed papers (n = 7). Furthermore, the pharmaceutical industry has been mentioned as the major industrial sector in 9 out of 13 cases. The selected articles were published between 2014 and 2026. As observed from the increasing trend of published articles starting from 2019, the

interest of scientists towards biotechnology applications of TW appears to be increasing. A comprehensive overview of the included studies is provided in Appendix 2.

NDM applications

Nicotine released into the environment as a result of tobacco industry activities has been classified as a hazardous chemical in the Toxic Release Inventory (TRI) by the U.S. Environmental Protection Agency, with concentrations in solid and liquid waste ranging from 3% to 6% (w/w). Due to its high-water solubility, nicotine leached from TW can easily migrate into groundwater. Numerous studies report that it has been detected with increasing frequency in leachate and runoff from landfills. Therefore, the development of effective methods for removing nicotine from the large quantities of waste generated by the tobacco industry has become imperative. In this context, a series of bioremediation processes have been developed to reduce the nicotine content in TW using more environmentally friendly approaches than conventional decontamination methods, while also recovering end products with added economic value (*10*).

Based on the biotechnological potential of TW and the ways in which it can be exploited, genetic engineering is rapidly advancing, with the aim of optimizing the metabolic pathways for nicotine degradation and recovering metabolites of interest produced by NDMs. In recent years, bioremediation based on NDMs, isolated enzymes, or whole cells used as biocatalysts has become a promising solution, and several strains have attracted attention due to their remarkable efficiency. These include primarily strains belonging to the genera *Pseudomonas* and *Agrobacterium*, known for their ability to decontaminate nicotine trough pyrrolidine and VPP pathways and produce bioactive compounds with applications in various industrial sectors. One of the major applications is the production of functional pyridines, used as precursors for the synthesis of various pharmaceuticals, including epibatidine analogues (an analgesic molecule), vitamin B6 (pyridoxine), analgesics (propiram, epibatidine), analeptics (nikethamide, camphotamide), and anti-inflammatory drugs (clonixin, nicoboxil). For example, compounds derived from nicotine metabolism that could be used directly in other applications include: 3-succinoyl-pyridine (SP) can be used as a precursor for the biosynthesis of anti-hypertensive agents (ω-heteroaroyl-(propynyl)-L-proline) (*10*), 6-hydroxy-3-succinoyl-pyridine (HSP) serves as a precursor for pioglitazone (for diabetes), eszopiclone (for insomnia), and crizotinib (for pulmonary cancer) (*11*), while 6-hydroxynicotine (6HN) can be used as a precursor for drugs designed to reduce symptoms of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (*12*).

Pseudomonas putida strains

The following studies propose the development of efficient biotechnological strategies for converting nicotine from TW into high-value chemical compounds such as 3-succinoyl-pyridine (SP) and 6-hydroxy-3-succinoyl-pyridine (HSP), using engineered strains of *Pseudomonas putida*.

The first study employs an *in vitro* approach based on enzymes previously expressed in *E. coli* and purified by chromatography. This enzymatic "assembly line" consists of NicAm, Pnao, and Sapd – from *P. putida* – for the sequential conversion of nicotine into SP. In this biocatalytic enzymatic cascade, NicAm (nicotine oxidoreductase) initiates the pathway by oxidizing nicotine to pseudooxynicotine (PN), which is then transformed into 3-succinoyl-semialdehyde-pyridine (SAP) by Pnao (pseudooxynicotine amine oxidase). The final step is catalyzed by Sapd (3-succinoyl-semialdehyde-pyridine dehydrogenase), which oxidizes SAP to produce the target product, SP. This sequence is assisted by an AKR module (aldehyde-ketone reductase) that ensures efficient and continuous regeneration of the $NADP^+$ cofactor required for the Sapd reaction, thereby maintaining a high catalytic flux. To optimize this process, the researchers employed spatial organization techniques through protein self-assembly using the

SpyCatcher/SpyTag system and their site-specific immobilization on agarose microspheres activated by biotinylation mediated by the AviTag-BirA system. The biotinylation method is essential because the BirA enzyme attaches a biotin as an anchor tag to the AviTag segment at a fixed and specific point (a lysine residue), allowing the enzymes to be anchored onto the streptavidin support and co-immobilized into agarose microspheres without damaging their structure or blocking their active sites. This proximity-based molecular architecture reduces the diffusion distance of metabolites between neighboring enzymes and increases the nicotine conversion rate to 63%, a value significantly higher than the 42.7% achieved by free enzymes. Ultimately, the system reached a yield of approximately 70% (obtaining 0.35 mM SP from 0.5 mM nicotine), representing a 1.6-fold increase in productivity compared to the non-immobilized system. Beyond efficiency, this biocatalyst offers exceptional thermal stability, retaining over 50% of its activity even after exposure to a critical temperature of 70°C, while free enzymes lose 90% of their capacity under the same conditions. The robustness of the system allows its reuse over eight consecutive cycles with activity retention above 60%, with laboratory tests confirming that over 87% of the enzymatic proteins remain stably bound to the agarose microspheres after repeated use (*13*).

The following two studies use whole cells as biocatalysts, where they were introduced into water containing nicotine or tobacco waste extracts (TWE) to produce essential chemical compounds, representing a sustainable and faster alternative to conventional chemical methods. In parallel with complex enzymatic systems, such as the study described above, this study focused on modifying the *P. putida* S16 strain to generate the P-HSP variant. By knocking out the *hspB* gene via homologous recombination, the degradation pathway of HSP into 2,5-dihydroxypyridine (2,5-DHP) was blocked. This approach allowed for the production of very large quantities of HSP, reaching 16.3 g/L from pure nicotine and 6.8 g/L using TWE, with performance 3.7 times higher than that of the wild-type strain. The efficiency was remarkable, with the P-HSP strain managing to convert 99.4% of the nicotine in just 5 hours, while the unmodified strain reached only 38.9% in the same time interval. This method offers several remarkable advantages: a rapid and simple reaction system consisting of nicotine-containing water as both the cell support and substrate, which facilitates faster purification of the target compound, as well as the ability to reuse the cells across multiple production cycles. All in all, these features make whole-cell biocatalysis a cost-effective, scalable, and environmentally friendly strategy for the biotechnological valorization of TW, with strong potential for industrial application in the pharmaceutical and green chemistry industries (*14*).

The third study details a sustainable approach for converting TW into SP using the genetically engineered strain *P. putida* S16Δ*spm*. This strain was obtained by deleting the *spmA* gene, which is responsible for the metabolism of SP into HSP, by homologous recombination. The study demonstrated the efficiency of the process through two distinct routes: strategy A, which uses a purified nicotine solution from TW to obtain a high-purity product with a crystal isolation yield of 54.2% relative to the initial nicotine content, and strategy B, which directly employs a raw tobacco suspension. The latter is considered a more environmentally friendly method as it eliminates the use of toxic organic solvents such as chloroform, relying instead on a simple water extraction at 60°C. Results obtained under fed-batch conditions showed titers of 9.8 g/L SP for strategy A and 8.9 g/L for strategy B, demonstrating that the process can be efficient even in complex media. A critical aspect of the optimization was pH control. While the optimal pH for pure nicotine was 9.0, maintaining a pH of 7.0 was necessary for the raw extract to prevent inhibition of the biocatalyst by complex tobacco components such as pectins and tannins. The whole-cell biocatalyst demonstrated remarkable stability, being successfully reused over three consecutive batch cycles with conversion yields of 97.0%, 100.0%, and 87.9%, respectively. This study highlights the industrial potential of *Pseudomonas* strain as a

robust cell factory capable of recycling TW and converting it into valuable precursors for vitamin B6, anti-hypertensive drugs, and analgesics such as epibatidine (*10*).

Agrobacterium tumefaciens strains

Research on the *A. tumefaciens* S33 strain focuses on the valorization of TW through the biotransformation of nicotine into high-value pyridine compounds.

The first study details an environmentally friendly route for the production of 6HN by blocking nicotine catabolism at this intermediate level, a process achieved by identifying and disrupting the *hno* gene that encodes the enzyme 6-hydroxynicotine oxidase, which catalyzes the oxidation of 6HN into 6-hydroxy-N-methilmyosmine (6NMM). This genetic modification, performed via homologous recombination using a suicide plasmid (pJQ200SK), generated the mutant strain S33-Δ*hno*, which lost its ability to further metabolize 6HN. In this case, whole mutant cells were used as a biocatalyst, a method applied exactly as with the previously described *Pseudomonas* strains. Under optimal conditions of 30°C and pH 7.0, the researchers achieved a molar conversion rate of approximately 98% and a specific catalytic rate of 1.01 g of product per hour per gram of dry cells. Under fed-batch conditions, the bioconversion of 4 g/L nicotine to 4.70 g/L 6HN was achieved. The final product was extracted with dichloromethane, yielding a recovery rate of 76.8% and serving as an essential precursor for the development of drugs for Alzheimer's and Parkinson's diseases (*12*).

The second study provides additional insight into the biochemical mechanism by identifying and characterizing Ald, an NAD-specific aldehyde dehydrogenase that was the last uncharacterized enzyme in the hybrid nicotine degradation pathway (VPP) in the *A. tumefaciens* S33 strain. This enzyme is involved in the conversion of 6-hydroxy-3-succinoyl-semialdehyde-pyridine to HSP. Its function was confirmed both by *in vitro* enzymatic assays using the SAP analog and by the deletion of the *ald* gene, which reduced the growth rate and biomass of the bacterium on nicotine-containing media. In contrast to similar enzymes in other strains that utilize NADP, Ald from the *A. tumefaciens* S33 strain is strictly NAD-dependent and exhibits remarkable versatility, possessing a broad substrate spectrum. In addition to nicotine metabolism intermediates, such as HSP, Ald can also efficiently metabolize other substances such as benzaldehyde, furfural, and acetaldehyde, exhibiting optimal activity at a pH of 9.0. To explore this potential, the *ald* gene was heterologously introduced into *E. coli* BL21 and DH5α strains, yielding recombinant variants such as Ald-N (equipped with a His tag at the N-terminus, proving to be more stable and active) and Ald-C (with a His tag at the C-terminus). The biotechnological application of this enzyme has been expanded through the use of recombinant *E. coli* cells expressing the *ald* gene, known as *E. coli*_Ald, which function as highly efficient biocatalysts. Using these, the efficient conversion of furfural to 2-furoic acid was achieved with a yield of 95.4% in just 2 hours, reaching 99% after 10 hours of reaction. These findings not only fully elucidate electron transfer and redox metabolism in nicotine degradation in *Agrobacterium*, but also provide new tools for the industrial catalysis of bio-based furan compounds used in the production of plastics and pharmaceuticals, a precursor for antibiotics, anti-hypertensive, and anti-parasitic drugs, thereby integrating toxic TW into a circular economy of renewable resources (*11*).

Novel NDM applications

Recent research has established that *Enterobacter hormaechei* ZS01, a bacterium isolated from cherry red tobacco, represents a new NDM strain identified for the first time with this capability, being able to efficiently catabolize nicotine through the simultaneous activation of the pyrrolidine pathway and a demethylation pathway. The strain was genetically modified to improve SP production, starting with the deletion of the *moaE* gene, involved in the biosynthesis of the molybdenum cofactor, to inactivate SpmABC monooxygenase, responsible for the conversion of SP to HSP, which generated the mutant strain ZS1 with a 2.78-fold higher

accumulation of SP compared to the wild-type strain. Subsequently, membrane permeability was optimized to facilitate nicotine absorption and SP release. This was achieved by modifying the structure of lipopolysaccharides through the deletion of the *rfaF* and *rfaC* genes, which encode heptosyltransferases responsible for the assembly of the core oligosaccharide layer. This genetic engineering strategy resulted in the ZS5 strain, which reached the highest SP concentration of 1.42 g/L in flask, while the triple mutant ZS8 (Δ*rfaC*Δ*rfaF*Δ*lpxM*), which further incorporates the deletion of the *lpxM* gene encoding a myristoyl transferase involved in lipid A biosynthesis, exhibited a 3.60-fold higher permeability but demonstrated a low yield due to the excessive disruption of cellular physiological functions and energy metabolism. In terms of yield, strain ZS1 demonstrated a nicotine conversion rate of 98.01% at concentrations of 1 g/L in liquid media and 95.80% in the TWE after 48 hours, maintaining remarkable tolerance at concentrations up to 5 g/L and adaptability across wide temperature (28 - 40˚C) and pH (5.5 - 8.5) ranges. Maximum performance was achieved in a 5 L bioreactor, where the use of strain ZS5 in fed-batch mode generated an SP titer of 4.11 g/L with a conversion rate of 68.82%, exceeding the previous values of 3.3 g/L reported for other NDMs. The biotechnological applications of this approach are significant, as the strain functions as a cellular factory that converts TW into precursors for anti-hypertensive drugs, compounds with anti-cancer, analgesic, and neuroprotective properties such as acetophenone (whose content increased by 391.20%), valuable substances for cosmetics, pharmaceuticals, and agriculture such as neophytadiene (98.78% increase), and monomers used in the manufacture of high-strength plastics and rubbers such as α-methylstyrene (an increase of 97.37%), thus providing a sustainable solution for the ecological management of TW (*15*, *16*).

Nicotine-tolerant non-NDMs

Research shows that some non-NDM strains possess remarkable resistance to nicotine, making them great candidates for producing valuable bioactive compounds.

An example of this microorganism is *Bacillus amyloliquefaciens* T4, an osmotolerant bacterium able to grow in the presence of nicotine and high osmotic pressure. Additionally, this microorganism can use TW as a nitrogen co-substrate to produce 2,3-butanediol (2,3-BD), a compound that has potential applications in the renewable fuel industry and in the production of bioplastics. The approach involves cellular biocatalysis in submerged fermentation at 37˚C and pH 7.0, yielding 1.54 g/L of 2,3-BD by using the soluble sugars naturally contained in TWE (≈ 3.07%) as a carbon source, while nicotine is tolerated and potentially utilized as growth factor, achieving yields of 30.06 g/L at 48 hours and 42.21 g/L at 72 hours when the process is supplemented with 200 g/L glucose as the primary carbon source and 50 g/L TWE as the nutrient source (*17*).

Similarly, the *Acetobacter xylinum* ATCC 23767 strain was used to produce bacterial cellulose (BC) using TWE as a substrate, which contains 25.1% sugars (glucose, fructose, sucrose) and 2.2% nicotine. Nicotine was removed from TW via distillation at pH 9.0, achieving a recovery rate of 88.7% - 90% without degrading the sugars necessary for BC synthesis. Implementing a two-stage fermentation over 16 days was essential: the first 7-day stage produced 2.27 g/L BC, but the decline in pH to 2.65 due to acid metabolism halted synthesis, even though more than half of the carbon sources remained unused. By harvesting the initial film to remove the oxygen transfer limitation and readjusting the pH to 6.5, the process was resumed, reaching a final yield of 5.2 g/L BC. BC is a biopolymer composed of glucose units joined by β-1,4-glycosidic bonds. It features a dense network of ultrafine fibers with properties superior to those of plant cellulose, including high purity, tensile strength, porosity, high water retention capacity, and thermal stability up to 250˚C. Due to its biological adaptability and mechanical characteristics, BC obtained from TW has wide-ranging applications, particularly in the textile industry (*18*).

Another approach focuses on the *Sinorhizobium meliloti* 224 strain, a symbiotic soil bacterium identified for its ability to produce cellulases (Avicelase and CMCase) through the bioconversion of TW. This bacterium was used both in submerged fermentation (SmF) and solid-state fermentation (SSF) conditions. In SmF, the optimal conditions were established at a concentration of 5 g/L of TW, an incubation time of 2 days, and a 9% inoculum, while the strain was optimized through acclimatization and successive passages on carboxymethyl cellulose media to promote metabolic adaptation, thereby achieving a roughly 3-fold increase in enzymatic activity. By chemically modifying the substrate with 1% NaOH, which increases the internal surface area and reduces the degree of polymerization and crystallinity of the lignocellulose, maximum yields of 1.503 U/g (Avicelase) and 1.615 U/g (CMCase) were achieved in SSF using a 10% acclimatized inoculum. These industrial enzymes, in which exoglucanase activity (Avicelase) is predominant, are fundamental for the bioconversion of TW rich in lignocellulosic material into useful products, with major applications in the textile, bioethanol, and animal feed industries (*19*).

At the end, the study on the *Bacillus subtilis* 168 strain demonstrates the transformation of a safe GRAS-certified microorganism (Generally Recognized as Safe) into an effective nicotine-removal system through the use of CRISPR-Cas9 genome editing technology. To create a functional metabolic pathway, the researchers integrated the nicotine oxidoreductase variant (*NicA2v*) and pseudooxynicotine amine oxidase (*Pppnao-S16*) genes – both derived from *P. putida* S16 – into the host genome to sequentially convert nicotine into SAP. To ensure cell viability and mitigate the metabolic stress caused by competition for FAD cofactors, the constitutive P43 promoter, which allows stable and controlled expression of the first enzyme. To manage the high toxicity of the second enzyme, a novel uORF translational attenuator (*KppqqA*), acting as a fine-tuning switch that limits protein production to an optimal level without compromising bacterial viability. System efficiency was further enhanced by introducing the *R54Q* mutation into the distal loop of the *Pppnao-S16* gene, which increased the reaction rate of the enzyme by 30% without altering substrate affinity, while the strategic integration of these genetic cassettes at the *LacA*, *LytC*, and *LytD loci* allowed for the simultaneous disruption of native autolysis genes, thereby significantly improving late-stationary-phase robustness and culture stability over time. The resulting strain, designated Bs.3.0, demonstrated the ability to degrade over 99% of nicotine within 24 hours and showed a 10-fold higher tolerance to this alkaloid compared to the wild-type strain. This system has key applications in the bioremediation of water, and soil, as well as in the development of probiotics for *in vivo* detoxification, protecting human health by preventing the formation of nicotine-derived carcinogenic compounds (*20*).

Co-culture systems

Recent research proposes an innovative approach to the valorization of TW by using co-culture systems, in which NDMs and non-NDMs are grown simultaneously to enhance environmental detoxification through the production of valuable metabolites, thereby overcoming the limitations of pure cultures where nicotine drastically inhibits the growth of productive microorganisms.

A highly efficient cellular method involves using the *Acetobacter oryzoeni* MGC-N8819 strain in co-culture with *Pseudomonas* sp. JY-Q/5Δ, an approach that eliminates the need for complex physico-chemical treatments to remove nicotine. In this system, the *Pseudomonas* strain was genetically modified by deleting five genes essential for the first step of glucose metabolism, transforming it into a selective NDM without consuming the glucose required by the first strain, thereby avoiding competition for the carbon substrate. The production yield for BC increased significantly in this co-culture system, reaching 4.61 g/L after 7 days, compared to only 3.27 g/L in the pure culture, and by applying a two-stage fermentation strategy, the final yield reached 6.00 g/L BC. The biotechnological applications of this cellulose are vast, the material

obtained exhibits thermal stability and superior mechanical properties, making it applicable in the textile industry (*21*).

Another advanced system of microbial co-culture consists in two strains: *Arthrobacter nicotinovorans*, and a metabolically engineered strain of *E. coli*, with the aim of achieving multi-metabolic conversion of tobacco dust. The method used is integrated cellular bioconversion, where the division of metabolic steps is clearly distributed: *A. nicotinovorans* ensures rapid detoxification of nicotine (approximately 430 mg/L) within 96 hours via the pyridine pathway, thereby protecting the sensitive strain of *E. coli*. The *E. coli* tMTScLS strain was genetically modified by co-transformation of two plasmids (pMVA1 and pScLS) containing genes for the mevalonate pathway and linalool synthesis – *Scmk* (mevalonate kinase), *Scpmk* (phosphomevalonate kinase), and *Scpmd* (mevalonate pyrophosphate decarboxylase) from *Saccharomyces cerevisiae*, and *Sahmgs* (HMG-CoA synthase) and *Sahmgr* (HMG-CoA reductase) from *Staphylococcus aureus*. The yield of this integrated system, optimized at an inoculation ratio of 3:1, allowed for the production of 8.3 g/L ethanol and 0.17 g/L linalool directly from the tobacco dust, demonstrating a synergy where the nicotine detoxification performed by *Arthrobacter* facilitates sugar metabolism and monoterpene synthesis by *E. coli* tMTScLS. The applications of this system are directly aimed at the circular bioeconomy through the sustainable production of ethanol, used as a biofuel, and high-value aromatic compounds, like linalool, which are widely used in the cosmetics, and pharmaceutical industries, providing a modular platform that can be adapted to produce other types of terpenoids or industrial flavors (*22*).

Discussion and Conclusions

This systematic review confirms that the microbial valorization of TW is a practical and promising way to support a circular economy. Most research focuses on two types of NDMs, *P. putida* and *A. tumefaciens*, which are representative strains for the pyrrolidine and VPP pathways, turning nicotine from TW into valuable products like functional pyridines. Interest in this field has grown significantly since 2019, following the discovery of new NDMs with extended biotechnological applications and the development of co-culture systems for the valorization of TW. At the same time, genome editing tools, such as CRISPR-Cas9, have made it possible to confer a new metabolic property related to nicotine degradation, thereby opening the road to applications that offer new perspectives for research.

Moving from the lab to a factory setting is the real hurdle. While these processes work in small flasks, scaling up to an industrial level is much more difficult. Because each lab uses different experimental conditions, it is extremely hard to establish a common protocol for a specific species to both decontaminate nicotine and produce valuable bioactive compounds at the same time. Additionally, compounds meant for medical use need to be tested outside the lab and validated through *in vivo* trials on animals or humans to prove they are safe and non-toxic.

The present systematic review is subject to several methodological limitations that must be acknowledged. First, the screening and data extraction process was conducted by a single author, which introduces a potential risk of bias in both study selection and interpretation. Furthermore, the exclusive inclusion of articles published in English since 2014 with an impact factor of IF ≥ 1.5 may have led to the exclusion of valuable research published in other languages, in lower-visibility journals, or prior to this timeframe. Additionally, due to the high degree of methodological heterogeneity regarding experimental conditions and bacterial strains, it was not possible to perform a quantitative meta-analysis. Consequently, a narrative synthesis approach was adopted, which is inherently more subjective in its nature.

The findings of this review carry significant implications for research, industrial practice, and public policy. Scientific efforts should focus on the transition of biotechnological processes from the laboratory to an industrial level while ensuring the safety and quality of the obtained

compounds. In the industrial sector, the application of these biotechnological methods could reduce environmental impact and create economic value from TW. Finally, the adoption of regulations that incentivize the valorization of industrial TW, in accordance with the 2030 Agenda and circular economy principles, could facilitate the extensive implementation of these sustainable solutions.

Declarations

Funding: This review received no external support;
Competing interests: The author declares none

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Appendix 1 – PRISMA 2020 Checklist

Section and Topic	Item#	Checklist item	Location where item is reported(*)
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	1-2
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	1-2
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	3
Information sources	6	Specify all databases, registers, websites, organizations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	2
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	2-3
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	3
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	3
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	3
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	N/A[1]
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	3
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	N/A[1]
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	3
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	N/A[1]

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Section and Topic	Item#	Checklist item	Location where item is reported(*)
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	N/A[1]
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	N/A[1]
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	N/A[1]
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	N/A[1]
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	N/A[1]
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	N/A[1]
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	4-5
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	4-5
Study characteristics	17	Cite each included study and present its characteristics.	4-5
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	N/A[1]
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimates and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	5-10
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	5-10
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	N/A[1]
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	N/A[1]
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	N/A[1]
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	N/A[1]
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	N/A[1]
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	10-11
	23b	Discuss any limitations of the evidence included in the review.	10-11
	23c	Discuss any limitations of the review processes used.	10-11

Section and Topic	Item#	Checklist item	Location where item is reported(*)
	23d	Discuss implications of the results for practice, policy, and future research.	10-11
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	N/A[1]
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	N/A[1]
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	N/A[1]
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	11
Competing interests	26	Declare any competing interests of review authors.	11
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Appendix 2

*the pages where the checklist items are located; [1]item applicable for meta-analysis.

Appendix 2 – Comprehensive overview of the included studies

Nr.	Author	Title	Year	Journal	Bacterial strain	Substrate	Bioactive Compound	Application
1	Shang *et al.*	An NAD-Specific 6-Hydroxy-3-Succinoyl-Semialdehyde-Pyridine Dehydrogenase from Nicotine-Degrading *Agrobacterium tumefaciens* Strain S33	2021	Microbiology Spectrum	*Agrobacterium tumefaciens* S33 and *E. coli*_Ald	Nicotine and furfural	6-hydroxy-3-succinoyl-pyridine and 2-furoic acid	Industrial biocatalysis; Green chemistry; Pharmaceutical and plastics industries
2	Lei *et al.*	Valorization of tobacco waste leaves: Discovery of *Enterobacter hormaechei* ZSS01for efficient nicotine degradation	2025	Biochemical and Biophysical Research Communications	*Enterobacter hormaechei* ZSS01	Nicotine from tobacco waste leaves	Acetophenone; Neophytadiene; α-methylstyrene	Food, cosmetics, pharmaceutical, and materials industries
3	Ye *et al.*	Bacterial cellulose production by *Acetobacter xylinum* ATCC 23767 using tobacco waste extracts as culture medium	2019	Bioresource Technology	*Acetobacter xylinum* ATCC 23767	Tobacco Waste Extracts	Bacterial cellulose	Textile industry
4	Wang *et al.*	Sustainable production of valuable compound 3-succinoyl-pyridine by genetically engineering *Pseudomonas putida* using the tobacco waste	2015	Scientific Reports	*Pseudomonas putida* S16Δ*spm*	Tobacco Waste	3-succinoyl-pyridine	Pharmaceutical industry
5	Yu *et al.*	Green strategy from waste to value-added-chemical production: efficient biosynthesis of 6-hydroxy-3-succinoyl-pyridine by an engineered biocatalyst	2014	Scientific Reports	*Pseudomonas putida* P-HSP	Tobacco Waste Extracts	6-hydroxy-3-succinoyl-pyridine	Industrial biocatalysis; Environmental protection and bioremediation; Pharmaceutical industry
6	Nie *et al.*	Improved bacterial cellulose production by *Acetobacter oryzoeni* MCG-N8819 in tobacco waste extract coupled with nicotine removal by *Pseudomonas* sp. JY-Q-5Δ	2025	International Journal of Biological Macromolecules	*Acetobacter oryzoeni* MCG-N8819 and *Pseudomonas* sp. JY-Q-5Δ	Tobacco Waste Extracts	Bacterial cellulose	Textile industry
7	Han *et al.*	Isolation and identification of an osmotolerant *Bacillus amyloliquefaciens* strain T4 for 2,3-butanediol production with tobacco waste	2022	Preparative Biochemistry and Biotechnology	*Bacillus amyloliquefaciens* T4	Tobacco Waste Extracts	2,3-butanediol	Pharmacy; Green chemistry; Agriculture; Industrial biocatalysis; Bioremediation

Nr.	Author	Title	Year	Journal	Bacterial strain	Substrate	Bioactive Compound	Application
8	Zhang *et al.*	Integrated microbial co-culture engineering for sustainable valorization of tobacco waste	2026	Journal of ecological Engineering	*Arthrobacter nicotinovorans* and *E. coli* tMTScLS	Tobacco Dust	Ethanol and linalool	Biofuel; Pharmaceutical and cosmetic industries
9	Buntic *et al.*	Cellulase production by *Sinorhizobium meliloti* strain 224 using waste tobacco as substrate	2019	International Journal of Environmental Science and Technology	*Sinorhizobium meliloti* strain 224	Tobacco Waste	Cellulase	Food, Biofuel and textile industries
10	Lei *et al.*	Targeted membrane permeability alterations in *Enterobacter hormaechei* ZS01 for high-level production of 3-succinyl-pyridine from tobacco waste	2025	World Journal of Microbiology and Biotechnology	*Enterobacter hormaechei* ZS01	Tobacco Waste	3-succinoyl-pyridine	Industrial biotransformation; Agrochemical manufacturing; Pharmaceutical industry
11	Wang *et al.*	Proximity-enhanced co-immobilized enzyme cascade for efficient bioconversion of nicotine to 3-succinoylpyridine	2026	Bioresource and Bioprocessing	*Pseudomonas putida*	Tobacco Waste	3-succinoyl-pyridine	Pharmaceutical industry
12	Yu *et al.*	Green route to synthesis of valuable chemical 6-hydroxynicotine from nicotine in tobacco wastes using genetically engineered *Agrobacterium tumefaciens* S33	2017	Biotechnology for Biofuels	*Agrobacterium tumefaciens* S33	Tobacco Waste	6-hydroxynicotine	Pharmaceutical industry
13	Li *et al.*	Synergic engineering of a GRAS-certified strain: Transforming *Bacillus subtilis* 168 into a high-performance nicotine scavenger	2026	Journal of Hazardous Materials	*Bacillus subtilis* 168	Nicotine	3-succinoyl-semialdehyde-pyridine	Environmental protection and bioremediation; Nicotine probiotic scavenger for human health