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Reviewer Article

Sustainable Hospital Wastewater Treatment Through Biopolymer-Immobilized Microorganisms: Opportunities and Challenges in Controlling Antimicrobial Resistance

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Abstract

Hospital wastewater represents a significant environmental reservoir of pharmaceutical contaminants, antibiotic-resistant bacteria (ARB), and antibiotic resistance genes (ARGs), presenting substantial challenges to environmental protection and antimicrobial resistance (AMR) control. This review critically examines the potential of biopolymer-immobilised microbial systems as sustainable alternatives for hospital wastewater treatment, emphasizing their functional mechanisms, treatment performance, and biosafety implications. Natural biopolymers, including alginate, chitosan, cellulose, and starch, are evaluated as microbial immobilization matrices capable of enhancing biomass retention, metabolic stability, and pharmaceutical biodegradation. Particular attention is given to the influence of matrix properties, immobilization strategies, and operational conditions on treatment efficiency. Critically, the review distinguishes antibiotic degradation from effective AMR mitigation, highlighting persistent challenges involving resistant microorganisms, extracellular ARGs, horizontal gene transfer, and microbial leakage. Emerging strategies involving engineered biopolymer composites, tailored microbial consortia, and integrated treatment technologies are discussed alongside limitations in scalability, economic feasibility, and environmental sustainability. Future priorities include standardized resistance monitoring, pilot-scale validation, and comprehensive biosafety assessment. Overall, this review provides a framework for developing scalable, environmentally responsible treatment systems that achieve measurable reductions in pharmaceutical contamination and AMR dissemination.

Keywords: Hospital wastewater; Biopolymeric materials; Microbial immobilization; Antibiotic biodegradation; Antimicrobial resistance; Environmental biosafety.

Introduction

Hospital wastewater has emerged as a critical environmental and public health concern due to its complex composition and its potential contribution to the dissemination of pharmaceutical contaminants, pathogenic microorganisms, and antimicrobial resistance (AMR) determinants [1]. Unlike conventional domestic wastewater, hospital effluents frequently contain heterogeneous mixtures of antibiotics, analgesics, disinfectants, cytotoxic compounds, diagnostic reagents, organic pollutants, and microorganisms originating from clinical activities, patient care, laboratories, and pharmaceutical consumption [2, 3]. The simultaneous occurrence of these contaminants creates a particularly challenging treatment environment, as chemical pollutants and biological hazards may interac
t in ways that influence microbial survival, pollutant persistence, and the selection of resistant populations [4]. Of particular concern is the continuous discharge of antibiotic residues and antibiotic-resistant bacteria (ARB), which may contribute to the environmental persistence and dissemination of antibiotic resistance genes [5]. Once released into receiving water bodies or municipal wastewater networks, these contaminants can interact with indigenous microbial communities, potentially facilitating the movement of resistance determinants across environmental reservoirs [6]. Consequently, effective hospital wastewater management has become increasingly relevant to the One Health framework, which recognizes the interconnected relationships among human health, environmental integrity, and microbial ecosystems [7].

Conventional hospital wastewater treatment generally relies on combinations of physical, chemical, and biological processes, including sedimentation, activated sludge treatment, membrane filtration,

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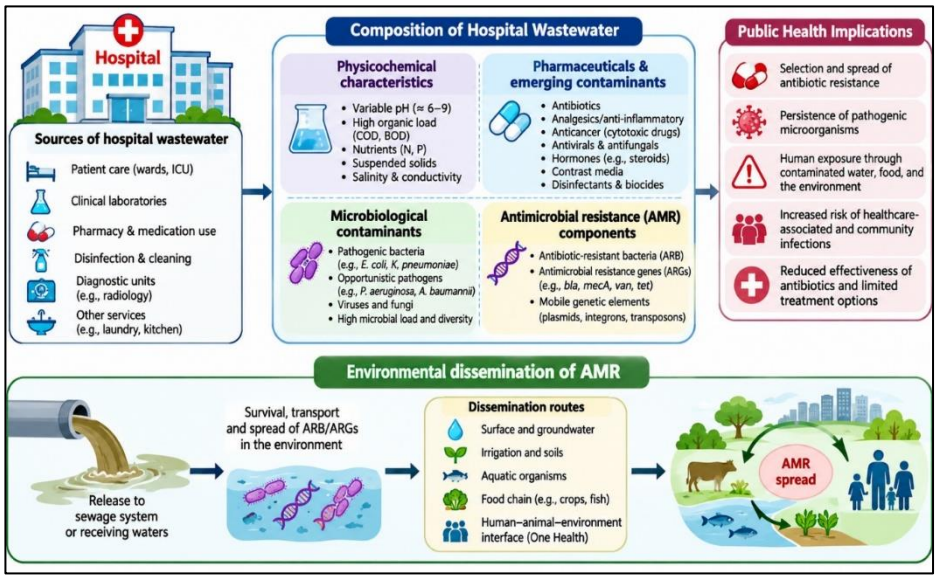
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adsorption, and chemical oxidation [8-11]. Although these technologies can substantially reduce organic matter, suspended solids, and selected microbial contaminants, their effectiveness against persistent pharmaceutical compounds and AMR-associated determinants varies considerably [12]. Microbial bioremediation has attracted considerable attention as a sustainable alternative or complementary strategy for wastewater treatment because of the ability of selected microorganisms to metabolize, transform, or immobilize a broad range of environmental pollutants [13]. Bacteria, fungi, yeasts, and microbial consortia possess diverse enzymatic systems capable of participating in the degradation of pharmaceutical residues, aromatic compounds, and other organic contaminants [14]. Nevertheless, the practical application of freely suspended microorganisms is frequently constrained by fluctuations in pollutant concentrations, environmental toxicity, hydraulic washout, limited biomass retention, and competition with indigenous microbial populations. Microbial immobilization has therefore emerged as a promising biotechnological approach for improving biomass retention, microbial stability, and treatment performance under challenging environmental conditions [15]. Within this context, naturally derived biopolymers have gained increasing interest as versatile immobilization matrices because of their renewable origins, tunable physicochemical characteristics, and potential environmental compatibility [16, 17]. The distinctive contribution of the present review lies in its integrated evaluation of biopolymer-immobilized microbial systems through the combined perspectives of materials engineering, environmental microbiology, pharmaceutical pollution control, and antimicrobial resistance management. Rather than examining microbial immobilization solely as a strategy for enhancing pollutant removal, this review critically considers how the physicochemical characteristics of biopolymeric matrices influence microbial viability, metabolic activity, contaminant transformation, resistance-associated microbial dynamics, and environmental biosafety. Particular emphasis is placed on the treatment of hospital wastewater containing antibiotic residues, pharmaceutical contaminants, ARB, and antimicrobial resistance genes (ARGs), while distinguishing evidence obtained from actual hospital effluents from findings derived from synthetic solutions or other wastewater sources.

Hospital Wastewater: Composition and Public Health Implications

Hospital wastewater represents a complex environmental reservoir containing diverse physicochemical pollutants, pharmaceutical residues, pathogenic microorganisms, and antimicrobial resistance determinants [18]. As illustrated in Figure 1, wastewater generated from patient care facilities, clinical laboratories, pharmaceutical activities, and hospital sanitation processes may contain antibiotics, cytotoxic compounds, disinfectants, ARB, and ARGs [19]. The discharge of inadequately treated hospital effluents into municipal sewage systems and receiving environments may facilitate the persistence and dissemination of resistant microorganisms and their associated genetic determinants [7]. Environmental pathways, including surface water, groundwater, agricultural soils, and aquatic ecosystems, may subsequently contribute to the circulation of antimicrobial resistance across interconnected human, animal, and environmental compartments. These interactions highlight the importance of adopting a One Health perspective when evaluating hospital wastewater contamination and developing sustainable treatment technologies to minimize potential environmental and public health risks [20].



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Figure 1. Sources, composition, and potential public health implications of hospital wastewater. Schematic representation of the major sources of hospital effluents and their physicochemical, pharmaceutical, and microbiological characteristics, including ARB, ARGs, and mobile genetic elements.

Hospital wastewater (HWW) is best understood not as a single homogeneous waste stream but as a variable mixture whose composition reflects the clinical services, patient load, and operational practices of the discharging institution. A recent critical review identified six major classes of pharmaceuticals and ten classes of antibiotics in HWW, with iodinated contrast media detected at concentrations up to 1050 µg/L the highest among reported emerging contaminants, while antibiotics showed the highest detection frequencies [21]. Approximately 30–90% of administered antibiotics are excreted unabsorbed and enter the effluent stream directly, creating a persistent selective pressure on microbial communities throughout the collection and treatment system [22]. Heavy metals, including mercury at concentrations ranging from 0.0024 to 0.019 mg/L, have also been reported, reflecting diagnostic and therapeutic uses [23]. The combined physicochemical and microbiological profile of HWW establishes it as a distinct environmental risk category. The coexistence of sub-therapeutic antibiotic concentrations, resistant bacterial populations, and mobile genetic elements creates conditions favouring horizontal gene transfer and the dissemination of resistance determinants beyond the hospital setting [24]. Ecotoxicological assessments indicate that sensitive organisms exhibit LC_{50} responses at HWW proportions as low as 0.508%–6.1% (v/v) over 12–96 hours of exposure, underscoring the potency of this effluent relative to its volume [25, 26]. These characteristics justify the position that HWW warrants source-directed management rather than reliance on dilution within municipal sewer networks, a theme developed in subsequent sections.

Hospital wastewater also represents a significant source of pharmaceuticals and emerging contaminants entering aquatic environments due to the extensive use of therapeutic agents, diagnostic chemicals, and disinfectants in healthcare facilities [27]. Among these contaminants, antibiotics are of particular environmental concern because of their biological activity, variable biodegradability, and potential contribution to the selection of antimicrobial resistance. The environmental persistence of pharmaceutical contaminants is strongly influenced by their molecular structures, physicochemical properties, and susceptibility to biological and chemical transformation [28, 29]. Certain compounds exhibit limited biodegradability and may remain detectable following conventional wastewater treatment, while others undergo partial transformation into metabolites with potentially persistent biological activity [30, 31]. Furthermore, the presence of multiple pharmaceutical substances and disinfectants can generate complex contaminant mixtures whose combined ecological effects remain insufficiently characterized. Antibiotic residues are particularly relevant because exposure to selective concentrations may favor resistant microbial populations, although the relationship between environmental antibiotic concentrations and resistance selection depends on microbial community composition and exposure conditions [32]. Emerging contaminants such as antimicrobial additives, quaternary ammonium compounds, and selected diagnostic chemicals may also influence microbial activity and wastewater treatment performance [33]. Figure 2 illustrates the spatial and temporal variability of bacterial communities across different water sources. At the phylum level (A), a limited number of dominant bacterial groups account for most of the community composition, although their relative abundances vary considerably among sampling sites. In contrast, the genus-level analysis (B) reveals substantially greater taxonomic diversity and pronounced fluctuations in bacterial community structure [34]. These findings highlight the heterogeneous nature of aquatic microbial communities and suggest that wastewater discharge, water source characteristics, and temporal environmental variations may influence bacterial community composition. The extensive use of antimicrobial agents in hospitals creates conditions in which resistant microorganisms may be enriched within clinical settings and subsequently introduced into wastewater through patient excretion, contaminated materials, and routine medical procedures [18]. Clinically relevant bacterial groups potentially encountered in hospital effluents include *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterococcus species [34]*. These microorganisms may carry resistance determinants associated with several antibiotic classes, including β-lactams, carbapenems, aminoglycosides, tetracyclines, and glycopeptides [35, 36]. Nevertheless, their occurrence and abundance vary considerably among healthcare facilities and depend on local antimicrobial consumption, infection prevalence, sanitation practices, and wastewater characteristics [37]. The magnitude of these effects depends on wastewater characteristics, treatment efficiency, environmental conditions, and the survival and ecological compatibility of introduced microorganisms.

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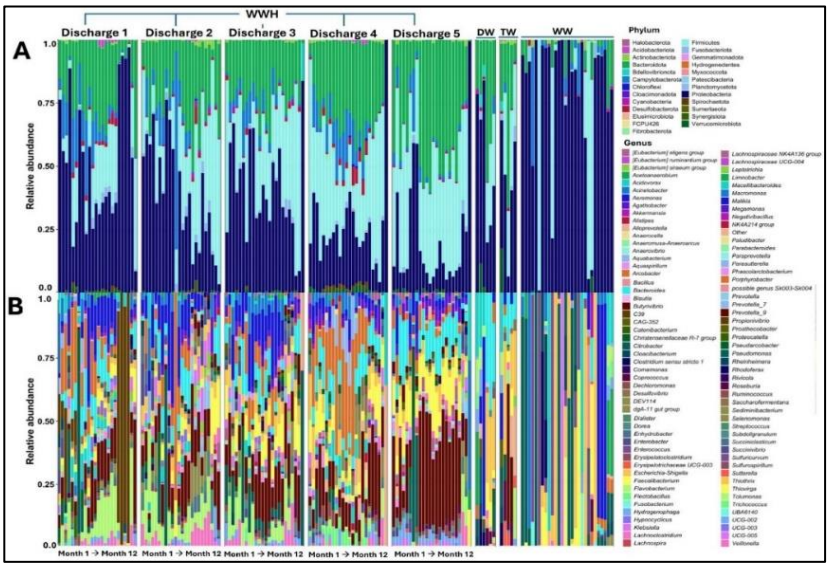
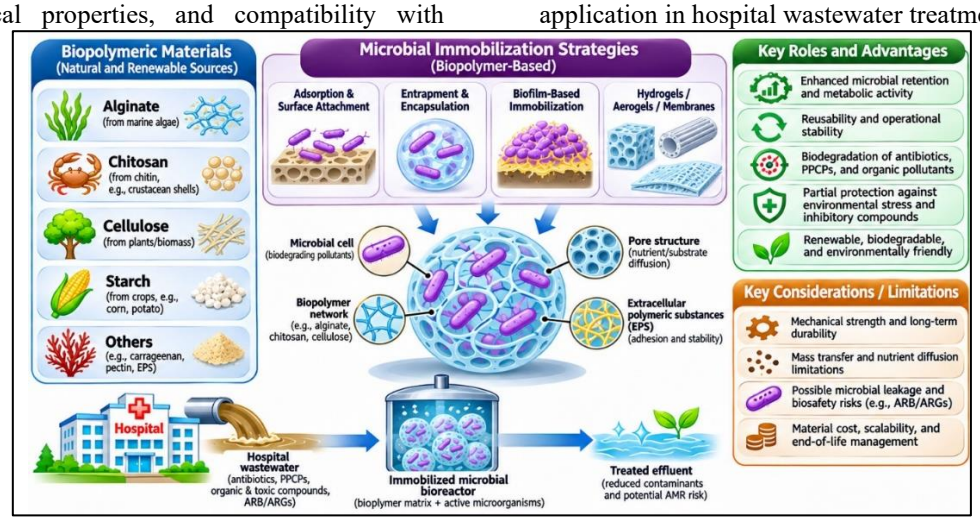


Figure 2. Temporal variations in the relative abundance and taxonomic composition of bacterial communities across five wastewater discharge sites in Hospital Juárez de México (Discharges 1–5), drinking water (DW), treated wastewater (TW), and wastewater (WW). Stacked bar charts illustrate bacterial community distributions at the (A) phylum and (B) genus levels. Different colours represent individual bacterial taxa, while variations in bar heights indicate their relative abundances across sampling locations and periods. Adapted with permission from [34]

Biopolymeric Materials for Microbial Immobilization

Biopolymeric materials have attracted considerable attention as sustainable and functional supporting matrices for microbial in environmental biotechnology and wastewater treatment [38, 39]. Their increasing relevance is associated with the growing demand for environmentally compatible treatment technologies capable of addressing complex mixtures of organic pollutants, pharmaceutical residues, and emerging contaminants. Unlike conventional synthetic immobilization materials, naturally derived biopolymers offer several potential advantages, including renewable availability, chemical versatility, tunable physicochemical properties, and compatibility with various microbial species [40, 41]. These characteristics make them particularly attractive for developing biologically active treatment systems in which microorganisms are retained within or attached to structured polymeric matrices, which include alginate, chitosan, cellulose, and starch, provide versatile matrices for microbial immobilization through adsorption, encapsulation, and biofilm formation [42, 43]. As illustrated in Figure 3, these materials can enhance microbial retention, metabolic stability, and the biodegradation of pharmaceutical and organic contaminants. However, matrix durability, mass-transfer limitations, microbial leakage, and biosafety considerations remain critical challenges for their application in hospital wastewater treatment.



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Figure 3. Biopolymeric materials and microbial immobilization strategies for hospital wastewater treatment. Schematic overview of major natural biopolymers, immobilization mechanisms, functional advantages, and key limitations associated with pollutant biodegradation and environmental biosafety.

Biopolymers used for microbial immobilization are primarily derived from natural polysaccharides, although proteins and other biologically sourced macromolecules may also be employed [44, 45]. Among the most extensively investigated materials are sodium alginate, chitosan, cellulose, starch, and their modified derivatives [46, 47]. These polymers contain functional groups, including hydroxyl, carboxyl, and amino groups, that influence water absorption, surface charge, crosslinking behavior, microbial adhesion, and interactions with dissolved contaminants [48]. Their physicochemical characteristics enable fabrication into different configurations, such as hydrogel beads, porous scaffolds, membranes, films, fibers, and aerogels [49]. The selection of a particular biopolymer and structural configuration depends on several factors, including the physiological characteristics of the immobilized microorganisms, the chemical properties of target pollutants, treatment conditions, and the intended operational lifetime [50]. Importantly, the suitability of a biopolymeric material should not be determined exclusively by its ability to retain microbial cells; it must also support adequate nutrient diffusion, preserve microbial metabolic activity, and maintain structural stability throughout wastewater treatment [51].

Alginate is among the most widely investigated natural polymers for microbial immobilization because of its relatively mild gelation conditions, hydrophilic characteristics, and ability to form three-dimensional networks through ionic crosslinking [52]. Alginate-based beads and hydrogels can provide hydrated microenvironments that support microbial metabolism while reducing biomass washout during repeated or continuous treatment operations [53, 54]. Their porous structure also permits the diffusion of dissolved nutrients and selected contaminants, although transport efficiency depends on polymer concentration, crosslinking density, bead dimensions, and environmental conditions [53, 54]. Alginate–chitosan composites, can form polyelectrolyte complexes through interactions between negatively charged alginate groups and positively charged chitosan groups under suitable conditions [55]. Such combinations may improve structural properties and microbial retention, although their effects on nutrient diffusion and microbial viability require experimental optimization. Accordingly, alginate-based immobilization should be evaluated not only for initial treatment efficiency but also for its ability to maintain structural integrity and biological functionality during prolonged exposure to authentic hospital effluents [56].

The practical application of biopolymeric immobilization materials depends on their economic feasibility, scalability, and environmental sustainability. Although naturally derived polymers are frequently described as renewable and biodegradable, their overall environmental performance is influenced by extraction methods, purification requirements, crosslinking agents, processing energy, and final disposal [57]. Table 1 presents Comparative characteristics of major biopolymeric materials used for microbial immobilization in wastewater treatment.

Table 1. Comparative characteristics of major biopolymeric materials used for microbial immobilization in wastewater treatment [58-60].

Biopolymer	Principal advantages	Major limitations	Potential immobilization applications
Sodium alginate	Mild gelation, hydrated structure, favorable microbial encapsulation	Swelling, ion exchange, limited mechanical durability	Hydrogel beads, encapsulation, composite matrices
Chitosan	Functional amino groups, film formation, surface modification	pH sensitivity, possible inhibition of immobilized microorganisms	Coatings, composite hydrogels, membranes
Cellulose and nanocellulose	Renewable availability, structural reinforcement, porous scaffold fabrication	Processing requirements, possible biological deterioration	Aerogels, porous scaffolds, membranes
Starch	Low cost, availability, biodegradability	Limited mechanical stability, swelling, microbial degradation	Composite beads, hydrogels, films
Carrageenan and pectin	Gel-forming capacity, hydrated matrices	Variable gel stability and mechanical properties	Entrapment beads, composite hydrogels
Biopolymer composites	Tunable mechanical strength, porosity, and surface chemistry	Greater formulation complexity, possible mass-transfer limitations	Multifunctional immobilization systems

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Microbial Immobilization Technologies and Mechanisms

Microbial immobilization in biopolymeric matrices represents a promising biotechnological approach for enhancing microbial retention, metabolic stability, and pollutant degradation in wastewater treatment systems [45, 58]. As illustrated in Figure 4, the principal immobilization strategies include adsorption and surface attachment, entrapment and encapsulation, biofilm-mediated immobilization, and the incorporation of microorganisms into engineered hydrogels, aerogels, and membranes. In hospital wastewater applications, these systems offer potential complementary treatment strategies, although reductions in pathogenic microorganisms and antimicrobial resistance determinants require specific experimental validation.

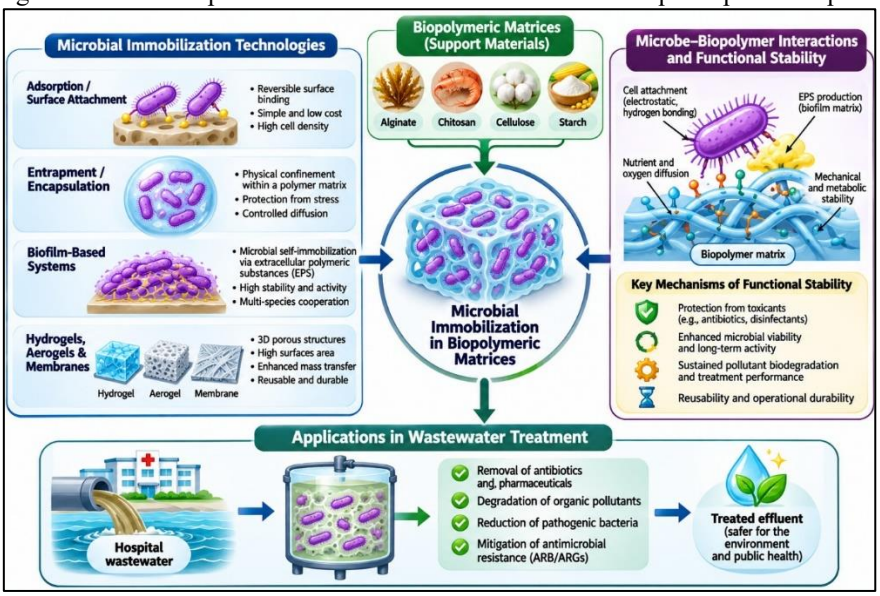


Figure 4. Microbial immobilization technologies, biopolymeric supporting matrices, and functional mechanisms relevant to hospital wastewater treatment.

Adsorption and Surface Attachment

Adsorption and surface attachment are among the simplest and most widely investigated approaches for microbial immobilization, involving the adhesion of microorganisms to solid supporting materials through physicochemical interactions [15, 61]. Unlike entrapment or encapsulation, these techniques generally do not require the complete confinement of microbial cells within a polymeric network. Instead, microorganisms attach to the external surfaces or accessible pores of supporting matrices through electrostatic attraction, hydrogen bonding, hydrophobic interactions, and interactions involving extracellular polymeric substances [62]. Natural biopolymers such as cellulose, chitosan, and alginate-based composites provide chemically versatile surfaces containing hydroxyl, amino, and carboxyl functional groups that can influence microbial adhesion [63, 64]. The efficiency of attachment depends on several factors, including microbial cell surface properties, polymer charge, surface roughness, porosity, pH, and ionic strength [65].

An important advantage of adsorption-based immobilization is the relatively direct accessibility of attached microorganisms to nutrients, oxygen, and target contaminants [66]. This characteristic can reduce some of the diffusion limitations associated with densely crosslinked immobilization matrices and support efficient microbial metabolism [67]. In hospital wastewater treatment, surface-attached microorganisms may contribute to the biodegradation of selected antibiotics, pharmaceutical residues, and biodegradable organic pollutants [68]. Furthermore, porous biopolymeric supports can provide increased surface area for microbial colonization, potentially enhancing biomass retention and facilitating continuous treatment operations [69, 70]. Surface modification and the incorporation of compatible functional materials may further improve microbial attachment and structural stability [71].

Adsorption-based immobilization presents limitations associated with the relatively weak or reversible nature of certain microbial–surface interactions [15]. Changes in hydraulic conditions, wastewater composition, ionic strength, and microbial growth patterns may promote cell detachment, potentially reducing treatment efficiency and increasing microbial release into treated effluents [72]. Such concerns are particularly relevant to hospital wastewater, where the unintended discharge of microorganisms carrying antimicrobial resistance determinants may create additional environmental risks. Moreover, excessive microbial accumulation can lead to biofilm overgrowth and reduced mass-transfer efficiency [73]. Consequently, the successful application of adsorption and surface attachment requires careful optimization of surface properties, microbial loading, and operating conditions [15]. Long-term investigations

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should assess not only pollutant removal efficiency but also microbial retention, community stability, and the potential dissemination of antibiotic-resistant bacteria during repeated or continuous treatment.

Entrapment and Encapsulation

Entrapment and encapsulation are established microbial immobilization strategies that involve physically confining microorganisms within biopolymeric structures while permitting the transport of nutrients, substrates, and metabolic products [74, 75]. Although these techniques are sometimes discussed interchangeably, entrapment generally refers to the incorporation of microbial cells throughout a polymeric network, whereas encapsulation involves their confinement within a defined compartment surrounded by a protective membrane or polymeric barrier [76]. Natural biopolymers, particularly sodium alginate, chitosan, and their composites, are commonly investigated because they can form hydrated matrices under relatively mild processing conditions [77]. Alginate is especially attractive due to its ability to undergo ionic gelation in the presence of divalent cations, allowing microbial cells to be incorporated into hydrogel beads without requiring harsh fabrication conditions [78].

The principal advantage of these approaches is their ability to improve microbial retention and provide a partially protective environment against unfavorable wastewater conditions. Entrapped microorganisms may exhibit greater resistance to hydraulic washout and selected environmental stresses compared with freely suspended cells [79]. Despite these advantages, the effectiveness of entrapment and encapsulation is strongly influenced by matrix architecture and mass-transfer characteristics. Highly concentrated or extensively crosslinked polymeric networks may restrict the diffusion of oxygen, nutrients, and pharmaceutical compounds, generating heterogeneous metabolic conditions within immobilized structures [80]. Microorganisms located near the matrix surface may therefore exhibit greater metabolic activity than those confined within deeper regions [81]. Conversely, matrices with excessive porosity or insufficient mechanical strength may experience microbial leakage, swelling, or structural deterioration during prolonged wastewater exposure [82].

Biofilm-Based Immobilization Systems

Biofilm-based immobilization represents a biologically driven approach in which microorganisms colonize supporting surfaces and develop structured communities embedded within self-produced extracellular polymeric substances [83]. These substances consist primarily of polysaccharides, proteins, extracellular DNA, and other biological macromolecules that contribute to microbial adhesion, structural organization, and environmental adaptation [84, 85]. Biopolymeric supports, including cellulose-based scaffolds, chitosan composites, and other functionalized natural polymers, can provide suitable surfaces for microbial colonization. Their surface chemistry, roughness, hydrophilicity, and pore structure influence the initial attachment of microorganisms and subsequent biofilm development [86]. The close association of microbial populations may facilitate metabolic cooperation, enabling different microorganisms to participate in sequential transformation pathways and the degradation of chemically diverse organic compounds [87]. Furthermore, the EPS matrix can influence local nutrient availability and provide partial protection against environmental stress, potentially improving microbial persistence and treatment stability [88].

These characteristics are particularly relevant to hospital wastewater, where pharmaceutical residues, disinfectants, and other inhibitory compounds may affect freely suspended microbial communities. Biofilm-associated microorganisms may therefore contribute to sustained biodegradation activity when suitable environmental and operational conditions are maintained [83]. However, biofilm formation introduces important limitations that must be considered when developing immobilized treatment technologies. Excessive biofilm accumulation may reduce effective pore space, restrict oxygen diffusion, and generate nutrient gradients that alter microbial metabolism [89]. Biofilm detachment and sloughing can also cause fluctuations in biomass concentration and potentially release microorganisms into treated effluents. More importantly, the close proximity of microorganisms and the presence of extracellular DNA within biofilm matrices may influence horizontal gene transfer, including the exchange of antimicrobial resistance determinants [90]. Although biofilm development does not invariably increase gene transfer, certain conditions may Favor interactions among resistant and susceptible bacterial populations.

Microbial Immobilization in Hydrogels, Aerogels, and Membranes

Biopolymer-based hydrogels, aerogels, and membranes represent versatile immobilization platforms that offer distinct structural and functional characteristics for microbial wastewater treatment [91, 92]. Hydrogels are three-dimensional, water-rich polymeric networks capable of retaining substantial quantities of water while maintaining a defined structure [93]. Their hydrated environments can support microbial viability and facilitate the diffusion of dissolved nutrients and contaminants, making them particularly suitable for microbial entrapment and encapsulation [94]. In hospital wastewater applications, hydrogel-based systems may provide controlled microbial retention and facilitate the biodegradation of selected pharmaceutical compounds. However, swelling, mechanical weakness, and diffusion limitations may reduce their operational durability [95].

Aerogels offer an alternative immobilization architecture characterized by highly porous, lightweight structures

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and potentially large accessible surface areas. Biopolymer-derived aerogels, particularly those prepared from cellulose, alginate, and related composites, can provide interconnected porous networks that may facilitate microbial attachment and contaminant transport [96]. Their structural characteristics create opportunities for integrating microbial biodegradation with adsorption-based contaminant removal. Nevertheless, the suitability of aerogels for microbial immobilization depends strongly on fabrication conditions and subsequent rehydration behavior [97]. Aerogel fragility, pore collapse, and structural deterioration under prolonged aqueous exposure may restrict their practical application. These limitations highlight the need for mechanically reinforced and water-stable aerogel systems designed specifically for biological wastewater treatment [98]. Biopolymeric membranes provide another promising platform by combining microbial immobilization with separation processes [99]. Microorganisms may attach to membrane surfaces or become incorporated into suitable membrane-associated structures, allowing biological transformation to occur alongside physical contaminant retention [100]. Such configurations may improve biomass management and facilitate integration with existing membrane-based treatment technologies. However, membrane fouling, biofilm overgrowth, hydraulic resistance, and microbial detachment remain significant operational challenges [101]. Moreover, membrane separation does not necessarily result in complete removal or inactivation of dissolved pharmaceuticals, extracellular resistance genes, or all microbial contaminants.

Antimicrobial Resistance Control and Biosafety

Antimicrobial resistance control and biosafety are critical considerations in the application of biopolymer-immobilized microbial systems for hospital wastewater treatment. Although these technologies offer promising opportunities for reducing pharmaceutical contaminants and potentially limiting the dissemination of antibiotic-resistant bacteria and resistance genes, concerns remain regarding horizontal gene transfer, microbial leakage, and pathogen persistence.

Antibiotic Resistance Genes in Hospital Wastewater

Hospital wastewater constitutes an important environmental reservoir of ARB, ARGs, and mobile genetic elements (MGEs), reflecting the intensive use of antimicrobial agents and the continuous discharge of microorganisms originating from healthcare activities [102]. Unlike conventional municipal wastewater, hospital effluents may contain elevated concentrations of clinically relevant antibiotics, disinfectants, pharmaceutical metabolites, and opportunistic pathogens, creating complex ecological conditions that can influence microbial community composition and resistance dynamics [18]. Resistance determinants associated with β-lactams, aminoglycosides, macrolides, tetracyclines, and fluoroquinolones have been detected in healthcare-associated wastewater, including genes such as *bla, mecA, tet*, and *van* variants [103]. Of particular concern are clinically important carbapenemase-encoding genes, including *bla*, which may occur in wastewater-associated Gram-negative bacteria [104]. Their environmental persistence is influenced by microbial host characteristics, wastewater composition, treatment conditions, and the genetic contexts in which these determinants are carried.

The environmental significance of ARGs extends beyond their detection because resistance determinants can occur within viable microorganisms, extracellular DNA, or mobile genetic structures capable of facilitating horizontal gene transfer under suitable conditions [105]. Plasmids, integrons, and transposons contribute to the organization, maintenance, and potential dissemination of resistance-associated genetic information across microbial populations [106, 107]. Furthermore, wastewater treatment environments may provide opportunities for interactions among clinically derived microorganisms and indigenous environmental bacteria, although the frequency and consequences of gene transfer depend on multiple ecological and molecular factors [108]. Antibiotic residues and other selective agents may additionally influence the relative abundance of resistant populations, particularly where exposure concentrations and microbial susceptibility favor resistance selection [109]. Nevertheless, detecting ARGs through molecular methods does not necessarily demonstrate the presence of viable pathogens, active gene expression, or direct transmission risks [110]. Consequently, comprehensive characterization of hospital wastewater resistomes requires approaches that extend beyond conventional culture-based detection. Quantitative polymerase chain reaction, shotgun metagenomic sequencing, and complementary culture-based analyses can provide information regarding ARG abundance, microbial community structure, and the potential association of resistance determinants with specific bacterial populations [111]. These methods are particularly relevant when evaluating emerging biopolymer-immobilized microbial treatment systems, where changes in resistance profiles must be interpreted alongside microbial viability, pollutant transformation, and environmental release.

Effects of Immobilized Microbial Treatment on Resistant Bacteria and Resistance Genes

Microbial immobilization within biopolymeric matrices has attracted increasing attention as a strategy for improving biological wastewater treatment through enhanced biomass retention, microbial stability, and sustained metabolic activity [112]. Immobilization may reduce microbial washout, improve resistance to fluctuations in environmental conditions, and facilitate repeated treatment cycles [15, 113]. These characteristics are particularly relevant to hospital wastewater, where

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complex contaminant mixtures and variable antibiotic concentrations can inhibit microbial metabolism and compromise conventional biological treatment performance [114]. However, the influence of immobilized microbial treatment on ARB and ARGs is considerably more complex than its effect on the removal of biodegradable organic pollutants.

The degradation of antibiotics by immobilized microorganisms may reduce the concentrations of antimicrobial compounds entering receiving environments, potentially modifying selective pressures acting on wastewater microbial communities [115]. Nevertheless, the removal of antibiotic residues does not necessarily result in a corresponding reduction in resistant bacterial populations or resistance gene abundance. Immobilized treatment systems may retain certain microorganisms within polymeric structures, but they can also provide protected microenvironments that support microbial persistence [116]. Moreover, the apparent reduction of ARGs in treated effluents may reflect physical retention, microbial inactivation, DNA degradation, or changes in community composition rather than complete elimination of resistance determinants. These mechanisms must be differentiated experimentally to establish whether a treatment system achieves meaningful reductions in environmental AMR dissemination potential. As emphasized in Figure 5, reductions in antibiotics, ARB, and ARGs should be regarded as separate performance endpoints, with their combined achievement requiring appropriate system design and experimental verification.

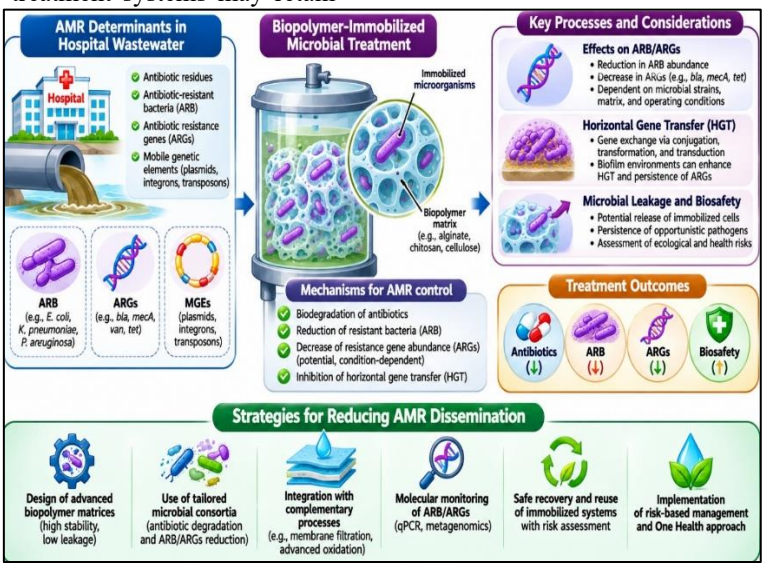


Figure 5. Antimicrobial resistance control and biosafety considerations in biopolymer-immobilized microbial systems for hospital wastewater treatment.

Horizontal Gene Transfer and Biofilm-Associated Resistance

Horizontal gene transfer (HGT) represents a fundamental mechanism contributing to the acquisition, diversification, and dissemination of antimicrobial resistance among bacterial populations [117]. HGT enables the exchange of genetic material between microorganisms through conjugation, transformation, and transduction. These processes are particularly relevant to wastewater environments, where complex microbial communities coexist with antibiotic residues, disinfectants, and other environmental stressors [118]. Conjugative plasmids, transposons, and integrons are important components of resistance-associated genetic mobility, although their respective roles differ in the acquisition, organization, and transfer of genetic determinants. Hospital wastewater may provide conditions that facilitate interactions between clinically derived resistant microorganisms and indigenous environmental bacteria, raising concerns about the potential circulation of medically important resistance genes beyond healthcare facilities [1, 119]. It has been reported that, gradients in oxygen availability, nutrient concentrations, and antimicrobial exposure can generate heterogeneous microbial microenvironments that influence stress responses and antibiotic susceptibility [120]. However, the relationship between biofilm formation and HGT is not universally positive; transfer frequencies depend on microbial species, genetic compatibility, biofilm architecture, and environmental conditions [120].

These considerations are particularly important for biopolymer-immobilized microbial systems, where the supporting matrix may promote microbial aggregation, surface colonization, and biofilm development. Although such characteristics can enhance biomass retention and pollutant degradation, they may also increase opportunities for interactions among microorganisms carrying resistance determinants [121]. Therefore, the assumption that microbial immobilization inherently

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suppresses antimicrobial resistance dissemination requires careful evaluation. Future investigations should assess how matrix composition, pore architecture, microbial community structure, and operational conditions influence conjugative transfer, extracellular DNA persistence, and the distribution of resistance-associated genetic elements. Integrating metagenomic analyses with experimental gene-transfer assays would provide a more comprehensive understanding of these interactions.

Strategies for Reducing Antimicrobial Resistance Dissemination

Effective mitigation of AMR dissemination from hospital wastewater requires treatment strategies that address antibiotic residues, ARB, and ARGs simultaneously. Biopolymer-immobilized microbial systems offer promising advantages through enhanced microbial retention, improved operational stability, and sustained biodegradation activity [122]. However, their effectiveness remains constrained by several interconnected limitations. Although composite reinforcement and polymer crosslinking can improve structural durability, excessive modification may compromise microbial viability, substrate accessibility, and biodegradability, creating a trade-off between material stability and biological performance [123]. More critically, antibiotic biodegradation is often incorrectly interpreted as evidence of AMR mitigation, despite the possible persistence of resistant microorganisms, transferable ARGs, and biologically active transformation products. Furthermore, immobilized microbial communities may create localized environments that facilitate microbial interactions and potentially horizontal gene transfer, particularly under residual antibiotic exposure [124]. This raises important biosafety concerns regarding the use of poorly characterized microbial consortia or strains carrying clinically relevant resistance determinants. Integrating microbial immobilization with membrane filtration, adsorption, advanced oxidation, or disinfection may overcome some limitations of standalone biological treatment; however, combining multiple technologies does not necessarily guarantee comprehensive AMR control [125]. For instance, disinfection may reduce viable ARB while leaving extracellular ARGs detectable, whereas advanced oxidation can transform antibiotics without ensuring complete detoxification or elimination of resistance-selective activity. A major weakness in the existing research is the reliance on short-term laboratory experiments and reductions in selected ARG markers as indicators of treatment success, with insufficient consideration of gene transferability, viable resistant populations, and long-term ecological risks.

Current Challenges and Future prospectives

Despite growing interest in biopolymer-immobilized microbial systems for hospital wastewater treatment, their transition from laboratory-scale investigations to practical implementation remains constrained by substantial technological, microbiological, and environmental challenges. One of the principal limitations concerns the structural instability of natural biopolymeric matrices under prolonged exposure to complex wastewater conditions [126]. Reinforcement through polymer blending, crosslinking, and composite fabrication may improve matrix durability; however, these modifications frequently introduce trade-offs involving reduced porosity, restricted nutrient diffusion, and impaired microbial metabolic activity [126]. Moreover, the performance of immobilized microorganisms is highly dependent on wastewater composition, hydraulic retention time, temperature, pH, and contaminant concentrations, making laboratory findings difficult to extrapolate to full-scale treatment systems.

Beyond material-related limitations, the most critical unresolved challenge is determining whether enhanced pharmaceutical biodegradation translates into meaningful reductions in AMR dissemination. Although immobilized microbial systems may effectively transform selected antibiotics, the persistence of ARB, ARGs, and biologically active transformation products can undermine their overall environmental benefits. Furthermore, immobilization matrices may provide protective microenvironments that favor microbial persistence and, under certain conditions, facilitate horizontal gene transfer among closely associated bacterial populations. This raises fundamental questions regarding the biosafety of immobilized microbial consortia, particularly when their resistance profiles and ecological interactions remain poorly characterized. Importantly, reductions in antibiotic concentrations or selected ARG abundances should not be interpreted as definitive evidence of AMR elimination, since resistance determinants may remain associated with viable microorganisms, extracellular DNA, or mobile genetic elements. Future investigations should therefore integrate microbial cultivation, quantitative PCR, metagenomic profiling, and functional assessments to distinguish contaminant degradation from actual reductions in resistance-associated environmental risks. Particular attention should also be directed toward evaluating the potential release of immobilized microorganisms and resistance determinants during matrix deterioration, biofilm detachment, or treatment-system failure. These considerations highlight the necessity of establishing standardized biosafety assessment protocols and incorporating downstream treatment barriers where biological processes alone cannot ensure adequate risk reduction.

From a technological perspective, integrating biopolymer-immobilized microbial systems with membrane filtration, adsorption, advanced oxidation, or disinfection represents a promising direction for addressing the limitations of individual treatment

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processes. Nevertheless, the additional energy requirements, operational complexity, and potential generation of secondary pollutants associated with hybrid technologies must be critically evaluated against their incremental treatment benefits. Future developments should emphasize multifunctional immobilization platforms capable of combining microbial biodegradation with contaminant adsorption and effective biomass retention, while avoiding unnecessary material complexity and excessive production costs. Equally important is the need for pilot-scale investigations comparing immobilized systems with conventional biological treatment under equivalent operating conditions, since evidence of superior performance, particularly regarding ARG removal and long-term stability, remains insufficient. Techno-economic analysis and life-cycle assessment should become integral components of technology development to evaluate material sourcing, fabrication energy, replacement frequency, waste management, and overall environmental sustainability. For resource-limited healthcare facilities, locally available biopolymers and simplified reactor configurations may offer practical advantages, provided that their effectiveness and biosafety are experimentally demonstrated. Ultimately, future progress should move beyond maximizing pharmaceutical removal efficiencies toward developing scalable, cost-effective, and biologically safe treatment systems capable of achieving sustained reductions in antibiotic residues, resistant microorganisms, and resistance determinants. Within the One Health framework, this transition is essential for establishing a stronger connection between wastewater treatment performance, environmental protection, and the prevention of AMR dissemination across interconnected human, animal, and aquatic ecosystems.

Conclusions

Biopolymer-immobilised microbial systems offer a promising and sustainable approach to hospital wastewater treatment by enhancing microbial stability, biomass retention, and pharmaceutical biodegradation. Natural biopolymers, including alginate, chitosan, cellulose, and starch, provide effective immobilization matrices; however, their performance depends on material properties, microbial activity, and operational conditions. Importantly, antibiotic removal does not necessarily ensure antimicrobial resistance (AMR) mitigation, as antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) may persist after treatment. Challenges involving matrix stability, microbial leakage, and horizontal gene transfer remain critical biosafety concerns. Future research should prioritize advanced biopolymer composites, integrated treatment technologies, standardized AMR monitoring, and pilot-scale validation using actual hospital wastewater. Techno-economic and life-cycle assessments are also essential to establish long-term feasibility and environmental sustainability. Overall, integrating treatment efficiency with comprehensive biosafety evaluation is crucial for developing scalable wastewater treatment technologies that minimize pharmaceutical pollution and AMR dissemination within the One Health framework.

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