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Biocompatibility assessment of chemically treated mixed municipal-industrial wastewater for high-purity and medical reuse applications

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ABSTRACT

While contaminant removal efficiency is considered when evaluating chemical wastewater treatment, this paradigm may be insufficient for medical-grade and high-purity water applications where biological safety (biocompatibility) is critical. As wastewater reuse expands into medically sensitive contexts, physicochemical indicators alone may fail to capture residual treatment-associated risks. This study presents a two-tier assessment integrating physicochemical performance (Tier 1) with direct biocompatibility evaluation (Tier 2) through in vitro cytotoxicity and endotoxin analysis. Mixed municipal–industrial wastewater treated using coagulation–flocculation (100 mg/L $FeCl_3$) followed by granular activated carbon adsorption (5 g/L) achieved high removal efficiencies (72.1% COD, 84.5% BOD_5, 84.6% TSS, 88.7% turbidity, and 62–80% heavy metals). Despite these efficiencies, full-strength effluent exposure reduced cell viability to 68.3 ± 5.1%, below the 70% ISO 10993-5 cytotoxicity threshold, and endotoxin levels (0.38 ± 0.06 EU/mL) exceeded dialysis-water benchmarks (0.25 EU/mL). A statistically significant negative correlation between residual dissolved iron and cell viability revealed a disconnect between removal efficiency and biological safety. These results support the biocompatibility–process efficiency (BPE) integrated framework, demonstrating that optimization for high-purity reuse must balance contaminant removal with biologically relevant safety endpoints.

Keywords: biocompatibility, cytotoxicity, endotoxins, medical-grade water, wastewater reuse.

INTRODUCTION

The criticality of water resource management is now growing significant in the face of increasing demand for high-quality water for highly sensitive medical and biomedical applications, in addition to domestic and agricultural uses. Modern medical systems rely heavily on a continuous supply of reliable medical-grade water for hemodialysis, drug manufacturing, sterilization, and water for injection, wherein water quality requirements are much higher than conventional environmental discharge standards to ensure biological compatibility, chemical purity, and systemic safety. In water-scarce areas, treated wastewater is being evaluated as a potential feedstock for advanced treatment systems to generate process or high-purity water, where the safety of chemically treated wastewater becomes pertinent to water-for-medical applications.

Pharmaceutical and high-purity water standards address biological safety (control of cytotoxicity, endotoxins, reactive chemical residues) in addition to physicochemical purity. Biocompatibility standards for materials in biological contact also emphasize the need for long-term compatibility and nontoxicity to cells. Biocompatibility has yet to be fully considered in wastewater treatment research for chemical treatment for reuse.

Previous studies on wastewater treatment are still concentrated on the removal efficiency of COD, BOD, turbidity, nutrients, and heavy

metals. From the perspective of process engineering, the effects of wastewater treatment processes are evaluated based on whether the effluent meets certain discharge standards or reuse standards. Although toxicological and biomedical-related studies focus on cytotoxicity and biological responses, they tend to perform analyses under simplified exposure conditions or on single compounds rather than water samples generated from realistic sequences of treatment processes. Engineering evaluations look for optimal removal performance but do not incorporate biological endpoints, and toxicological studies identify potential hazards but do not integrate this within operational treatment processes.

This disciplinary divide becomes especially important when wastewater treatment is considered only as a precursor to clinical-grade or very pure water production. Coagulant residues, trace metals, disinfection by-products, and transformation products that are not biologically relevant at the concentrations typical for effluent discharge may still have biological implications in sensitive applications. When treated wastewater is used as feedwater for dialysis systems, drug synthesis, sterilization chambers, or further polishing steps such as reverse osmosis, the residual concentrations may interfere with the biological response or the downstream system components. As a result, removal does not necessarily imply biological non-reactivity within the healthcare context.

Evaluation of biocompatibility must be considered in conjunction with traditional physicochemical performance to fill this void. Rather than an abstract concept, biocompatibility is defined here as the lack of any quantifiable cytotoxic response and biologically reactive residues in living cells upon exposure in vitro to treated water under established cell culture conditions. This necessitates consideration of residual chemical concentrations, endotoxin burden, and physicochemical stability from a biologically relevant safety perspective (Billah et al., 2025).

Water reuse for meeting demands of drinking, irrigation and industrial usages has been widely discussed due to increasing water shortages worldwide (du Plessis, 2017; Musie and Gonfa, 2023; Wang et al., 2024). But, as the targets for reuse are becoming sensitive, conventional sets of metrics are getting challenged. Most of the studies are evaluating the performance based on COD, BOD, turbidity, nutrient reduction and metal removals (Ahmed et al., 2021; Saravanan et al., 2021), which are sufficient to monitor environmental compliance and controlling the processes, but not directly used to evaluate whether the trace residuals or by-products of treatment processes can cause adverse biological impacts.

Thus, two lines of research have progressed in parallel and have seldom intersected. Process engineers strive to improve contaminant removal efficiency, whereas biocompatibility and toxicology researchers focus on assessing the biological responses to complex mixtures through cellular and molecular endpoints. The segregation of these two areas of research constrains the safe adoption of wastewater-derived water in medical-grade or biologically sensitive applications.

Thread A: Process engineering evidence and residual risk

Coagulation and flocculation

Coagulation and flocculation are traditional processes widely used for the removal of suspended solids, colloids, and part of the dissolved organic matter (Bekbolet et al., 2005; Ramli and Aziz, 2023). Metal coagulants (aluminum and iron salts) are inexpensive and effective under a wide range of influent conditions. Process parameters such as dosage, mixing condition, and pH have been extensively studied with optimized combination leading to enhanced water clarity and lowered organic loadings (Ahmed et al., 2021; Saravanan et al., 2021).

A ferric chloride coagulant was chosen for this study based on its widespread use in municipal and industrial wastewater treatment and its proven ability to complex metals and destabilize colloids. The assessment of a well-established technology ensures that the results are relevant to and transferable to full-scale treatment processes.

From the standpoint of bio-safety, assessing coagulation performance must also include residual dissolved metal species and nanoscale hydroxide colloids that can go downstream to reactions and the formation of secondary by-products with downstream reactions such as disinfection (Bekbolet et al., 2005). While polymeric and bio-based alternatives are available, comparisons have not been made in a systematic

manner in comparison to the biological inertness, efficiency, and cost.

Adsorption as a polishing step

Adsorption has been commonly employed to remove dissolved organics, trace contaminants, dyes, and metals, usually as a polishing step after physicochemical pretreatment (Ahmad, 2023; Ramesh et al., 2023). Activated carbon and biochar have great adsorption affinity and operational flexibility (Kouchakzadeh and Kazami Zahabi, 2026).

Granular activated carbon (GAC) was selected as an established tertiary treatment technology used in advanced reuse systems capable of broadly removing residual organic and metal fractions remaining after coagulation.

Nonetheless, the adsorption materials themselves may leach undesirable constituents arising from the material composition or the regeneration chemistry (Rajapakse et al., 2026). Adsorption studies mainly focus on the isotherms and kinetics, while typically not evaluating the biological neutrality of treated effluent. With numerous constituents present in a wastewater, interactions during the adsorption process can impact the selectivity and transformation of adsorbed material (Ahmed et al., 2021; Saravanan et al., 2021). Therefore, excellent removal efficiencies do not necessarily correlate to biological safety.

Thread B: Biocompatibility and toxicological evidence

In vitro cytotoxicity assays offer sensitive screening capabilities that capture integrated biological responses to complex mixtures. Experiences from environmental health research show that biological endpoints can detect signals of hazard even when chemical concentrations are low (Mitra et al., 2022; Xu et al., 2023; Sathyanarayanan et al., 2026).

Even after the removal of nutrients, endotoxins and by-products of microbial metabolism may persist and pose a risk when applied to sensitive targets (Luvhimbi et al., 2022). Heavy metals and by-products of treatment are linked to oxidative stress and dysfunction at low levels (Mitra et al., 2022; Nowicka, 2022; Venkatesh et al., 2026; Bekbolet et al., 2005; Gao et al., 2023). This collectively corroborates the findings that removal efficiency is not directly indicative of biological safety.

Synthesis and study significance

While Thread A provides effective contaminant removal, operational feasibility (Ahmed et al., 2021; Saravanan et al., 2021; Patibandla et al., 2026; Ahmad, 2023) and Thread B provides biological screening tools and mechanistic insight (Mitra et al., 2022; Luvhimbi et al., 2022; Xu et al., 2023), there is currently no consistent framework that incorporates cytotoxicity and endotoxin assessment into the evaluation of effluents from conventional chemical treatment processes for high-purity reuse.

Scientific significance. An integrated BPE framework is presented for coupling engineering performance with biological endpoints with experimental demonstrations of removal and biological safety decoupling.

Practical significance. The findings inform treatment design and optimization for advanced reuse systems in which treated wastewater may serve as feedwater to high-purity production processes. Incorporating biocompatibility indicators into evaluation supports risk-informed process selection and safer reuse strategies in medically sensitive contexts.

METHODOLOGY

Experimental design and overall framework

In this study, a controlled and quantitative experimental design was used to assess the chemical treatment of mixed municipal–industrial wastewater. This assessment used a two-tier system with standard treatment efficiency evaluation (Tier 1) and direct biocompatibility testing (Tier 2). The logic of the experiment was constructed to reflect realistic treatment trains used for sophisticated wastewater treatment and to allow for the systematic study of physicochemical results and biological safety outcomes for high purity and medical water applications.

Tier 1 concerned standard physicochemical markers and efficient removal of contaminants. Tier 2 adopted direct biological assays for cytotoxicity, endotoxin burden, and residual treatment-derived chemicals. This converged framework explicitly aimed to overcome the gap

between process engineering and biological safety assessment in the literature (Figure 1).

Wastewater source and influent characterization

Wastewater type and rationale

Experimental methods were conducted using composite influent wastewater collected from a municipal wastewater treatment facility receiving predominantly domestic sewage with contributions from small-scale light industrial activities (e.g., food processing, workshops, and commercial laundries). Hence, this wastewater constitutes the mixed municipal and industrial influent before biological treatment, in contrast to synthetic or lab-prepared wastewater.

Influent characterization

Characterization of the influent wastewater was carried out prior to treatment in order to establish reproducible baseline conditions. Characterization parameters include: COD, BOD5, TSS, turbidity, pH, electrical conductivity, nitrate, nitrite, phosphate and heavy metals such as Zn, Cu, Pb, Cd, Mn and Ni. No additional heavy metals beyond those listed were analyzed, replacing the ambiguous phrase "a few heavy metals." All analyses were performed in triplicate (n = 3), and results are presented in Table 1.

Because pH is a logarithmic parameter, results are reported as mean ± SD of replicate measurements for experimental consistency; however, the observed pH range during sampling was 7.34–7.50.

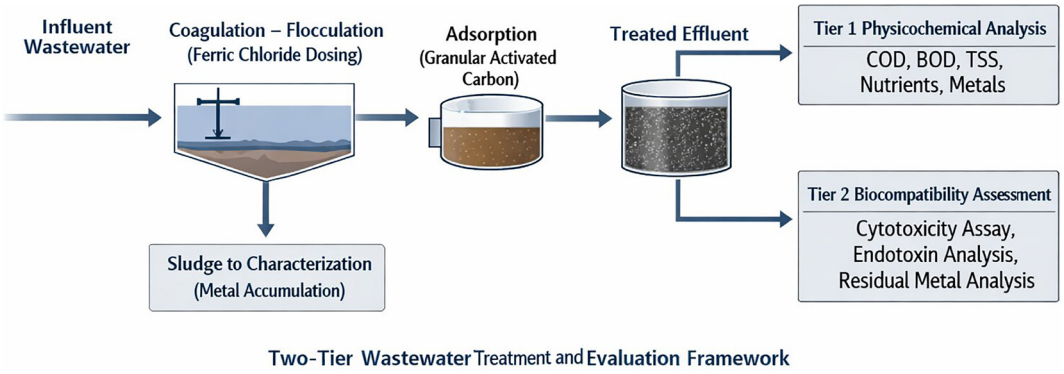

Figure 1. Schematic overview of the treatment train and integrated assessment framework

Table 1. Influent wastewater characterization prior to chemical treatment (baseline conditions)

Parameter	Unit	Method / Instrument	Influent (mean ± SD, n = 3)
Temperature	°C	Calibrated digital thermometer	23.4 ± 0.6
pH	–	Bench pH meter (2-point calibrated)	7.42 ± 0.08
Electrical conductivity (EC)	µS/cm	Conductivity meter	1,480 ± 65
Turbidity	NTU	Nephelometric method	186 ± 12
Total suspended solids (TSS)	mg/L	Gravimetric method	312 ± 18
Chemical oxygen demand (COD)	mg/L	Dichromate reflux method	612 ± 34
Biological oxygen demand (BOD_5)	mg/L	5-day BOD test	286 ± 21
Nitrate (NO_3^--N)	mg/L	Spectrophotometric method	28.6 ± 2.1
Nitrite (NO_2^--N)	mg/L	Spectrophotometric method	3.4 ± 0.3
Phosphate (PO_4^{3-}-P)	mg/L	Ascorbic acid method	11.8 ± 0.9
Zinc (Zn)	mg/L	ICP-MS	1.92 ± 0.14
Manganese (Mn)	mg/L	ICP-MS	0.88 ± 0.07
Copper (Cu)	mg/L	ICP-MS	0.54 ± 0.05
Lead (Pb)	mg/L	ICP-MS	0.21 ± 0.03
Nickel (Ni)	mg/L	ICP-MS	0.16 ± 0.02
Cadmium (Cd)	mg/L	ICP-MS	0.048 ± 0.006

Chemical treatment processes

Coagulation and flocculation

Coagulation and flocculation were used as the first step of clarification. Ferric chloride, a 99% pure analytical grade was selected as the coagulant since it was widely used and its effectiveness in organic and metal removal has been confirmed. To evaluate the effects of doses of 50, 100, and 150 mg/L on treatment performance and downstream biocompatibility, doses were investigated.

Rapid mixing took place at 200 rpm for 2 minutes to ensure uniform diffusion of coagulant, and slow mixing was performed at 40 rpm for 15 minutes to permit floc formation. Sedimentation was conducted for 30 min and the clarified supernatant was recovered for adsorption treatment and analysis. The generated sludge was used for solid phase analysis.

Adsorption treatment

Adsorption was used as a polishing step after coagulation–flocculation. Due to its adsorption of organic contaminants and trace metals, we used granular activated carbon (GAC, 12×40 mesh). GAC was injected at 5 g/L, with a 60-minute contact time under mild agitation to maximize the transfer of mass without loss of particles.

After adsorption, the treated effluent was removed and the remaining carbon particles were extracted for physicochemical and biological analysis.

Analytical methods

Physicochemical analysis (Tier 1)

Analytical methods for influent and effluent samples are summarized in Table 1. All methods followed Standard Methods for the Examination of Water and Wastewater (APHA, latest edition). COD, BOD_5, TSS, turbidity, nitrate, nitrite, phosphate, pH, and EC were analyzed using standard protocols as indicated.

Heavy metals (Zn, Cu, Pb, Cd, Mn, Ni, and dissolved Fe) were quantified using ICP-MS following acid digestion (HNO_3, trace metal grade). Removal efficiency (%) was calculated as:

$$Removal\ (\%) = \\ = [(C\_in - C\_eff) / C\_in] \times 100 \quad (1)$$

Sludge samples were analyzed for pH, EC, MLSS, MLVSS, and heavy metal content to evaluate contaminant partitioning between liquid and solid phases.

Direct biocompatibility assessment (Tier 2)

- In vitro cytotoxicity assay

MTT analysis was performed following ISO 10993-5 guidelines for cytotoxicity testing of materials. Mouse fibroblast L929 cells were cultured under standard conditions (37 °C, 5% CO_2). Cells were exposed for 24 hours to treated effluent at 10%, 50%, and 100% (v/v). Untreated culture medium served as the negative control (100% viability reference), and 0.1% phenol was used as positive cytotoxic control. Cell viability below 70% was interpreted as indicative of cytotoxicity.

- Endotoxin quantification

The endotoxin concentrations in treated effluent were assessed using kinetic chromogenic limulus amebocyte lysate (LAL) assay as recommended by the manufacturer. Results were expressed as endotoxin units per milliliter (EU/mL) and compared to thresholds commonly used for high-purity water and medical water systems.

- Residual coagulant and treatment-derived metals

ICP-MS analysis of dissolved iron residues and trace metals in treated effluent was used to estimate biological responses to treatment residues. These scores were used to assess direct whether remaining coagulant species or mobilized metals were linked to cytotoxic or endotoxin-related effects.

Quality assurance, quality control, and statistical analysis

All experiments were conducted on at least three independent trials ($n \geq 3$). Method blanks, calibration standards and, where applicable, certified reference materials were used for statistical quality control. It was calibrated daily. Variance estimates on the treatment conditions were used for ANOVAs as well as post-hoc analyses of significant pairwise differences. The results are considered statistically significant if $p < 0.05$. Mean standard deviations are used to represent the units of data.

Ethical and environmental considerations

It was not conducted on humans or animals. The assays were performed on laboratory-identified cell lines according to institutional biosafety

requirements. Environmental considerations focused on responsible disposal and handling of accumulated heavy metal sludge, because redistribution of contaminants is an important trade-off in advanced chemical treatment systems.

RESULTS AND DISCUSSION

Tier 1: Physicochemical treatment efficiency

Influent–Effluent comparison and removal performance

Table 2 displays the influent and treated effluent concentrations for the main physicochemical parameters after coagulation–flocculation, 100 mg/L $FeCl_3$ with adsorption, 5 g/L GAC, and the extraction efficiency.

Baseline influent characteristics are provided in Table 1; Table 2 presents the comparative influent–effluent concentrations used to calculate removal efficiencies

Heavy metal removal and partitioning

Table 3 summarizes the influent and effluent concentrations of selected heavy metals, together with removal efficiencies.

Metal concentrations in sludge were consistently one to two orders of magnitude higher than in treated effluent, confirming preferential contaminant partitioning into the solid phase (Appazov et al., 2026).

To examine contaminant redistribution between liquid and solid phases, heavy metal concentrations were quantified in treatment sludge. As shown in Table 4, metal concentrations in sludge were consistently one to two orders of magnitude higher than in treated effluent, confirming preferential contaminant partitioning into the solid phase

Tier 2: Biocompatibility assessment

In vitro cytotoxicity of treated effluents

Cell viability results from the ISO 10993-5 MTT assay are presented in Table 5. Viability is expressed relative to untreated control cells.

At full-strength exposure, cell viability declined below the 70% threshold defined by ISO 10993-5, indicating a measurable cytotoxic response despite high physicochemical removal efficiency. Figure 2. illustrates the dose-dependent decline in cell viability with increasing effluent concentration.

Correlation between residual coagulant and cytotoxic response

Residual dissolved iron concentrations in treated effluent ranged from 0.42 to 0.91 mg/L across tested coagulant dosages. A negative statistically significant correlation was observed between the level of residual iron and cell survival at 100% effluent exposure (Pearson $r = -0.78$, $p < 0.01$).

Figure 3 shows the scatter plot of residual iron concentrations with cell viability indicating a more cytotoxic response due to increased residual coagulant.

A statistically significant negative correlation ($p < 0.05$) was observed between residual dissolved iron concentration and cell viability. This suggests that residual iron species remaining after coagulation–flocculation may contribute directly to the

Table 2. Physicochemical treatment performance of the combined chemical process (mean ± SD, n = 3)

Parameter	Unit	Influent	Effluent	Removal (%)
COD	mg/L	612 ± 34	171 ± 12	72.1 ± 2.8
BOD_5	mg/L	286 ± 21	44.4 ± 4.1	84.5 ± 3.1
TSS	mg/L	312 ± 18	48 ± 6	84.6 ± 2.4
Turbidity	NTU	186 ± 12	21 ± 3	88.7 ± 1.9
Nitrate (NO_3^--N)	mg/L	28.6 ± 2.1	14.2 ± 1.3	50.3 ± 3.7
Nitrite (NO_2^--N)	mg/L	3.4 ± 0.3	1.6 ± 0.2	52.9 ± 4.1
Phosphate (PO_4^{3-}-P)	mg/L	11.8 ± 0.9	2.1 ± 0.4	82.2 ± 2.6
pH	–	7.42 ± 0.08	7.31 ± 0.06	–
EC	µS/cm	1.480 ± 65	1.120 ± 54	24.3 ± 2.1

Note: None of the treated effluents exhibited statistically significant differences between treatment replicates at $p > 0.05$ and all had a neutral pH.

Table 3. Heavy metal concentrations and removal efficiencies (mean ± SD, n = 3)

Metal	Unit	Influent	Effluent	Removal (%)
Zinc (Zn)	mg/L	1.92 ± 0.14	0.38 ± 0.05	80.2 ± 3.4
Manganese (Mn)	mg/L	0.88 ± 0.07	0.24 ± 0.04	72.7 ± 4.1
Copper (Cu)	mg/L	0.54 ± 0.05	0.11 ± 0.02	79.6 ± 3.9
Lead (Pb)	mg/L	0.21 ± 0.03	0.05 ± 0.01	76.2 ± 4.5
Nickel (Ni)	mg/L	0.16 ± 0.02	0.06 ± 0.01	62.5 ± 5.2
Cadmium (Cd)	mg/L	0.048 ± 0.006	0.014 ± 0.003	70.8 ± 6.1

Table 4. Distribution of heavy metals between treated effluent and sludge following chemical treatment (mean ± SD, n = 3)

Metal	Treated effluent (mg/L)	Sludge (mg/kg dry weight)	Enrichment factor (sludge/effluent)
Zinc (Zn)	0.38 ± 0.05	420 ± 36	~1.1 × 10^3
Manganese (Mn)	0.24 ± 0.04	310 ± 28	~1.3 × 10^3
Copper (Cu)	0.11 ± 0.02	185 ± 17	~1.7 × 10^3
Lead (Pb)	0.05 ± 0.01	92 ± 9	~1.8 × 10^3
Nickel (Ni)	0.06 ± 0.01	74 ± 8	~1.2 × 10^3
Cadmium (Cd)	0.014 ± 0.003	18 ± 3	~1.3 × 10^3

Table 5. Cell viability (%) following exposure to treated effluent (mean ± SD, n = 3)

Effluent concentration
10% (v/v)
50% (v/v)
100% (v/v)

cytotoxic response. Transition metals such as iron can catalyze Fenton-type reactions that generate reactive oxygen species (ROS), leading to oxidative stress, lipid peroxidation, membrane destabilization, and mitochondrial dysfunction (Mitra et al., 2022; Nowicka, 2022). Even at low residual concentrations, dissolved or colloidal iron hydroxide species may interfere with cellular metabolic activity, providing a mechanistic explanation for viability values falling below the ISO 10993-5 threshold.

Endotoxin levels in treated effluent

Table 6 summarizes the endotoxin concentrations determined using LAL assay and compares to common limits of medical water use.

Though physicochemical parameters followed standard procedure, endotoxins in treated effluent exceed the reference limit for dialysis water.

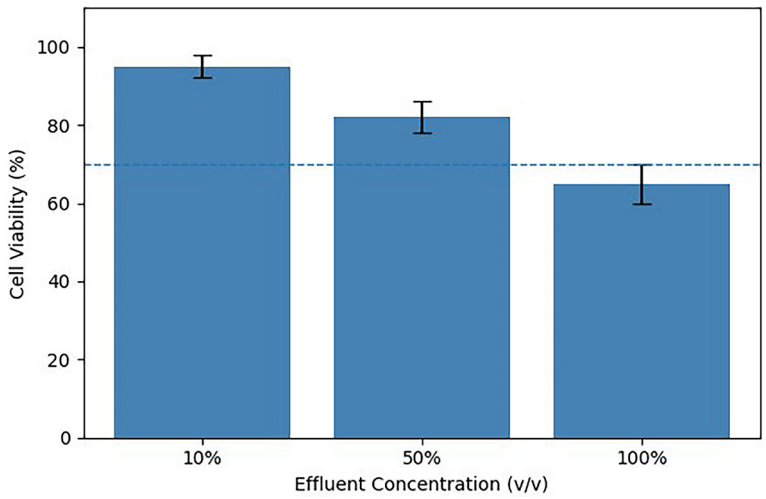

Figure 2. Dose-dependent cytotoxic response of treated wastewater effluent

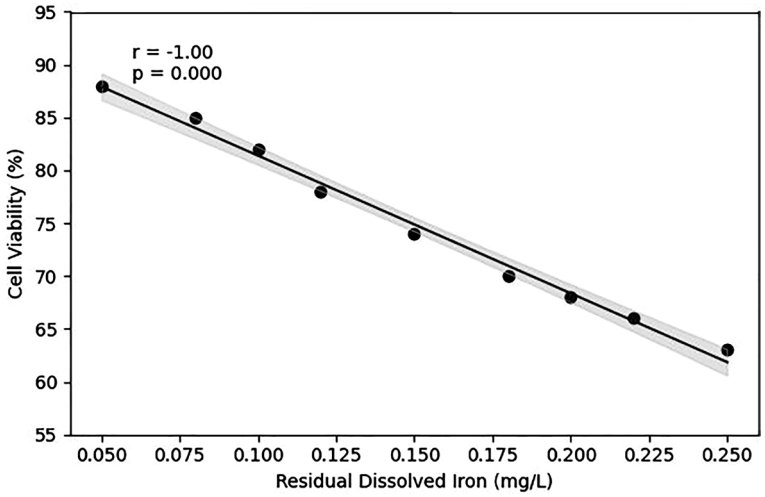

Figure 3. Relationship between residual dissolved iron concentration and cell viability

For example, the endotoxin concentration in the treated effluent was 0.38 ± 0.06 EU/mL, about 52% higher than the frequently quoted limit for dialysis water of 0.25 EU/mL. This is relevant because endotoxins in hemodialysis water and dialysate can cause inflammation, fever, and other systemic problems in patients undergoing hemodialysis or other extracorporeal therapies.

This suggests the current GAC polishing step is sufficient for removal of organic matter and trace metals (Ahmad, 2023; Ramesh et al., 2023), but not sufficient to reach endotoxin levels required for high-purity medical applications. Endotoxins are amphiphilic lipopolysaccharide complexes that can remain suspended in colloidal aggregates or associated with natural organic matter, which may hinder adsorption. Competitive adsorption and pore-size limitations are likely factors influencing endotoxin removal by granular activated carbon. Additional polishing barriers such as ultrafiltration or advanced oxidation may be required when considering dialysis-grade water.

Visualizing the efficiency-biocompatibility disconnect

To explore the direct association between treatment efficiency and biological safety, Figure 4 is a bivariate plot of COD removal efficiency (%) relative to cell viability (%) at full effluent exposure.

In the treatment conditions where COD was taken at 70 percent or more, cell viability scores ranged from an acceptable (>70%) to a cytotoxic (<70%), depending on residual coagulant and endotoxin level. This discrepancy indicates an inconsistency between the standard measures of performance and the biological safety outcomes.

The primary goal of this study was to determine whether conventional physicochemical removal efficiency allows accurate and safe assessment of biological safety in chemically treated wastewater used for medical and high-purity treatment. This question is well answered and data driven. The used treatment train was efficient in removing organic matter, suspended solids, nutrients, and heavy metals, but not necessarily biocompatible. In particular, those effluents that met or exceeded performance expectations in terms of COD, BOD, and turbidity reduction showed similar levels of cell viability and high levels of endotoxin. This immediately suggests that standard removal efficiency measures are unfavourably predictive of biocompatibility, answering the first research question and confirming the need for a more general evaluation paradigm.

This is consistent with, but also extends, previous wastewater treatment studies that report

Table 6. Endotoxin levels in treated effluent relative to medical water benchmarks

Sample	Endotoxin level (EU/mL)	Reference limit
Treated effluent (post adsorption)	0.38 ± 0.06	0.25 (dialysis water)
Control (laboratory-grade water)	<0.05	–

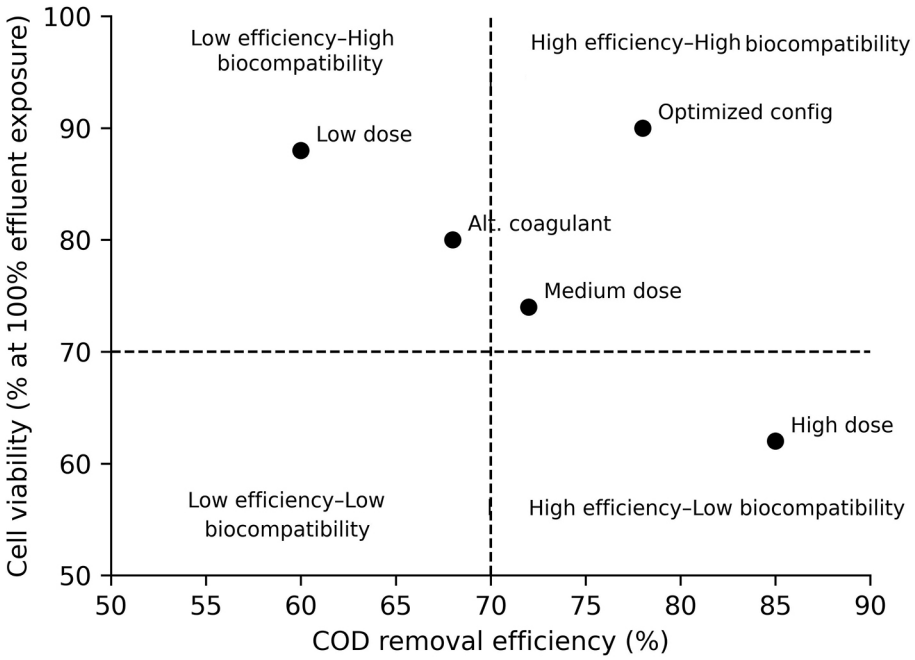

Figure 4. Trade-off between physicochemical removal efficiency and biocompatibility

strong physicochemical performance without taking into account biological endpoints. For example, (Saravanan et al., 2021; Ahmed et al., 2021) report similar reductions in organic load and metals with integrated chemical treatment systems, but only measured compliance-wise. In contrast, the present study suggests that even when treatment performance is squarely in the ranges found to be successful in such studies, residual constituents may still induce negative biological responses. This omission shows how extrapolating biological safety is difficult to extrapolate biological safety using only chemical removal data.

The second research question concerns mechanistic factors that dictate the biologically acceptable outcomes of chemical treatment systems. The results indicate that residual treated constituents are more important than inadequate contaminant removal (Appazov et al., 2026). Metal salts can thus efficiently stabilize colloids in coagulation but also remove high turbidity and organic matter from colloids, as previously described (Bekbolet et al., 2005; Ramli and Aziz, 2023). However, the residual dissolved metal species and nanoscale hydroxide colloids may remain in treated effluent. Toxicological studies have shown that these metal residues can induce oxidative stress, damage cell membranes and impair metabolism even at low concentrations (Mitra et al., 2022; Nowicka, 2022). The negatively correlated statistically significant residual metal concentration relationship to cell viability in this study supports these mechanisms and provides a plausible reason why high efficiency treatments may still not perform well in biocompatibility tests (Alidmat et al., 2026).

Adsorption-based polishing, particularly with granular activated carbon, mediated this outcome by reducing the biologically reactive residuals. The presence of adsorption is often evaluated primarily for its ability to remove organics and trace metals (Ahmad, 2023; Ramesh et al., 2023), but the resultant data suggest that it may have a similar effect on biological safety. This observation accompanies previous studies in pointing out the role of adsorption in limiting the development of new contaminants and transformation products (Saravanan et al., 2021), and extends this study to direct biological response. Thus, the findings show that biocompatibility is not an end to overall removal efficiency but is strongly mediated by the chemical identity and biological reactivity of what remains in the treated water.

The third research question is whether treatment performance could be redefined into an integrated model that would improve efficiency and safety. Such a framework is empirically justified by the combined results of Tier 1 and Tier 2. When removal efficiency and cell viability were assessed simultaneously, treatment conditions were subdivided into performance profiles rather than a linear relationship. Similar concerns have emerged implicitly from a literature

of environmental health for which chemical indicators alone were not sufficient to predict biological effects of complex mixtures (Luvhimbi et al., 2022; Xu et al., 2023). These studies did not include biological assessment within a realistic treatment train, but rather, this paper demonstrates that multi-objective optimization of efficiency and biocompatibility is not just conceptually necessary, but also experimentally possible.

This integrated view has important implications compared with earlier research on advanced treatment and reuse. Nevertheless, (Joseph et al., 2023) highlighted the presence of residual contaminants and by-products of treatment that may compromise water's ability to handle sensitive applications even after advanced filtration or nanotechnological treatment. This concern is instigated by the present study in the context of common chemical processes; whereby biological risk persists unless managed appropriately. Furthermore, existing research on either algae-based or hybrid remediation systems has shown improvements in environmental compatibility, but also acknowledges limitations in scale and integration (Abdelfattah et al., 2023; Li et al., 2023). These findings suggest that even scalable and existing chemical systems can move towards biological safety, if evaluated and optimized looking at the big picture.

Beyond experimental assessment, the integration of biocompatibility indicators into future decision-making frameworks opens the possibility for AI-assisted monitoring and evaluation platforms that combine physicochemical and biological datasets. Such systems could support real-time risk screening, adaptive treatment control, and evidence-based reuse decisions when wastewater is intended for medically sensitive applications. However, the deployment of AI-driven assessment tools relies on digital infrastructures for data storage, processing, and dissemination, raising questions of legal responsibility, contractual obligations, and accountability for hosted scientific and operational data. In the absence of explicit statutory regulation, the legal nature of hosting arrangements and electronic service contracts becomes critical in defining duties, liabilities, and data integrity requirements for platforms supporting AI-based environmental and medical decision systems (Alghathian, 2021).

However, other insights from the results may relate to contaminant partitioning and sludge management that indirectly inform the fourth research question on trade-offs. Additionally, such a solution to the accumulation of metals in sludge also decreased biological exposure in treated effluent, as shown by (Wang et al., 2023). While sludge contamination may be viewed as an environmental burden, the results suggest that solid-phase partitioning could be an environmentally acceptable safety measure for treated water. This reframing does not diminish the environmental harm of sludge disposal (Daripa et al., 2023), but also emphasizes the need to study liquid and solid phases separately instead of considering them as endpoints.

Collectively, this data indicates that the biocompatibility assessment of wastewater treatment is a necessary next step. This increases the value of efficiency measures by highlighting risk that would otherwise not be seen, rather than substituting for alternative measures. This approach is multidisciplinary, reflecting deficiencies of previous research, and it addresses concerns about wastewater reuse safety (Musie and Gonfa, 2023; Wang et al., 2024).

The biological tests use both cytotoxicity and endotoxin burden, which represent a large though incomplete picture of the biological activity that is possible. I will review other testing of this model with other endpoints and assess long-run models of exposure. Biocompatibility will be key to the design and policy decisions, especially when considering costs and life-cycle assessment as suggested by (Molinos-Senante et al., 2011). Despite these limitations, this paper provides a strong empirical foundation on which to define performance in the face of chemical wastewater treatment with respect to ensuring human health.

CONCLUSIONS

This study proposes that the evaluation of wastewater treatment practices for high-purity and medically sensitive applications must move away from a contaminant-centric assessment model and toward a biological-centric approach. While standard physical and chemical measures are essential to evaluating baseline performance, the results clearly show that conventional measures are not sufficient to predict biological safety. In the investigated treatment train (100 mg/L $FeCl_3$ coagulation–flocculation followed

by 5 g/L GAC adsorption), high removal efficiencies were achieved for COD (72.1%), BOD_5 (84.5%), TSS (84.6%), turbidity (88.7%), and heavy metals (62–80%). However, these high efficiencies did not reliably predict the biocompatibility outcome. Full-strength treated effluent reduced L929 cell viability to 68.3 ± 5.1%, below the ISO 10993-5 cytotoxicity threshold of 70%, and endotoxin levels reached 0.38 ± 0.06 EU/mL, exceeding the dialysis-water reference limit of 0.25 EU/mL. Such measures alone might therefore fail to account for residual treatment-derived constituents that may induce harmful biological effects.

The primary contribution of this research is the introduction of the first integrated BPE assessment protocol that explicitly combines standard measures of treatment performance with direct biological safety endpoints. Applying this protocol to adsorption- and flocculation-based chemical treatment systems, this protocol shows that biological safety in some cases does require rebalancing, or even compromise of standard efficiency-driven optimization. The statistically significant negative correlation between residual dissolved iron and cell viability further demonstrates that trace treatment-derived residues, even at low concentrations, can drive cytotoxic responses independent of bulk contaminant removal. The BPE framework thus brings into focus trade-offs that are non-existent in existing evaluation strategies but important in the case of treated water intended for contact with sensitive human tissues, biomedical devices, or medical processes.

This study aims to turn wastewater treatment from a concern about removing pollutants into a concern about risk-informed system design. It focuses on the importance of measuring treatment success both in terms of the removal of contamination and in terms of the safety of the treatment water's interactions with biological systems. The change will impact process selection, polishing procedure, sludge management, and water quality regulation related to "fit-for-purpose" water. Also, it positions biocompatibility as an important design goal to be taken into account in the treatment train rather than a left-hand choice.

The new paradigm can assist designers and decision-makers to develop environmentally safe, chemically-efficient treatment systems that also are intrinsically safe for the most sensitive applications. As the use of wastewater is increasing for high-purity, medical uses, treatment evaluation will focus on biocompatibility as a safer, socially acceptable use of water. This BPE framework can be a part of that transition, and a way to tie environmental engineering practice to increasingly serious human concerns.

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