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Optimization of Granulation Mechanisms in UASB Reactors for Hospital Wastewater Treatment: An FTIR-Based Approach

Optimisation des mécanismes de granulation dans les réacteurs UASB pour le traitement des eaux usées hospitalières : une approche basée sur la spectroscopie FTIR

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ABSTRACT :

This study compared a control UASB reactor (R1) with an optimized reactor (R2) receiving mineral and organic additives for the treatment of hospital wastewater from the University Clinics of Kinshasa. Granules were characterized by Fourier-transform infrared (FTIR) spectroscopy, and relationships between spectral features and treatment indicators were explored using Pearson correlations, multiple linear regression, and one-way ANOVA. Compared with R1, R2 granules showed higher relative intensities in bands assigned to O–H/N–H, C–H, Amide I, Amide II, COO^-, C–O–C, carbonate, and Fe–O/silicate groups. The descriptive data also showed lower residual COD (42 ± 4 vs 210 ± 15 mg/L), BOD_5 (31 ± 3 vs 120 ± 10 mg/L), and TSS (15 ± 2 vs 95 ± 8 mg/L) in R2, together with higher Fe (91 ± 3% vs 60 ± 6%) and Cu retention (96 ± 2% vs 55 ± 5%). FTIR intensities were strongly associated with residual organic-pollution indicators and metal retention, and the reported regression model identified amide- and carbonate-related bands as predictors of COD. The reported ANOVA results indicated differences between R1 and R2 ($p < 0.001$). These findings support an association between enhanced bio-mineral granule structuring and improved reactor performance. However, the manuscript does not report the number of independent replicates, exact additive doses, ANOVA degrees of freedom, or regression diagnostics; these elements should be provided before the inferential results are considered fully reproducible.

Keywords: UASB granules; FTIR spectroscopy; extracellular polymeric substances (EPS); mineralization; hospital wastewater treatment; heavy metals.

RESUME :

Cette étude a comparé un réacteur UASB témoin (R1) à un réacteur optimisé (R2) recevant des additifs minéraux et organiques pour le traitement des eaux usées des Cliniques universitaires de Kinshasa. Les granules ont été caractérisés par spectroscopie infrarouge à transformée de Fourier (FTIR), et les relations entre les caractéristiques spectrales et les indicateurs de traitement ont été explorées par corrélations de Pearson, régression linéaire multiple et ANOVA à un facteur. Comparativement à R1, les granules de R2 présentaient des intensités relatives plus élevées pour les bandes attribuées aux groupements O–H/N–H, C–H, Amide I, Amide II, COO^-, C–O–C, carbonate et Fe–O/silicates. Les données descriptives montraient également, dans R2, des concentrations résiduelles plus faibles de DCO (42 ± 4 contre 210 ± 15 mg/L), de DBO_5 (31 ± 3 contre 120 ± 10 mg/L) et de MES (15 ± 2 contre 95 ± 8 mg/L), ainsi qu'une rétention plus élevée du Fe (91 ± 3 % contre 60 ± 6 %) et du Cu (96 ± 2 % contre 55 ± 5 %). Les intensités FTIR étaient fortement associées aux indicateurs résiduels de pollution organique et à la rétention des métaux; le modèle de régression rapporté identifiait notamment les bandes amides et carbonates comme prédicteurs de la DCO. Les résultats d'ANOVA rapportés indiquaient des différences entre R1 et R2 ($p < 0,001$). Ces résultats soutiennent une association entre une structuration bio-minérale accrue des granules et de meilleures performances du réacteur. Toutefois, le manuscrit ne précise pas le nombre de répétitions indépendantes, les doses exactes d'additifs, les degrés de liberté de l'ANOVA ni les diagnostics de régression; ces éléments doivent être fournis avant de considérer les résultats inférentiels comme pleinement reproductibles.

Mots clés : granules UASB ; spectroscopie FTIR ; substances polymériques extracellulaires (EPS) ; minéralisation ; eaux usées hospitalières ; métaux lourds.

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I. INTRODUCTION

The management of hospital wastewater represents a major challenge at the intersection of public health, environmental protection, and water resource management. These effluents are characterized by high chemical and microbiological complexity, including organic pollutants, pharmaceutical residues, pathogenic microorganisms, heavy metals, and antibiotic-resistant bacteria. Such contaminants contribute to environmental pollution, antimicrobial resistance, and ecological toxicity in receiving aquatic systems (Verlicchi et al., 2019; Oliveira et al., 2023; Singh et al., 2024).

In many tropical and resource-limited regions, wastewater treatment infrastructure remains inadequate. Conventional aerobic and physico-chemical systems often suffer from high operational costs, energy demand, and limited efficiency in removing emerging pollutants. These limitations are exacerbated by fluctuating organic loads, temperature variability, and restricted access to technical resources, making the implementation of sustainable treatment solutions particularly challenging.

Anaerobic technologies, particularly Upflow Anaerobic Sludge Blanket (UASB) reactors, have emerged as promising alternatives for treating high-strength wastewater. These systems allow efficient organic matter degradation and biogas production with relatively low energy requirements. Their performance largely depends on the formation of dense and stable anaerobic granules composed of microbial consortia embedded in extracellular polymeric substances (EPS). This matrix plays a critical role in microbial aggregation, structural cohesion, and stabilization of metabolic interactions essential for methanogenesis (Sheng et al., 2010; Liu et al., 2020).

Granule formation and stability are influenced by several operational and environmental factors, including organic loading rate, hydraulic retention time, pH, temperature, and nutrient availability. Recent studies have highlighted the beneficial role of mineral and organic additives, such as carbonates, biochar, or clay, in enhancing granule density, microbial adhesion, and heavy metal adsorption capacity (Chen et al., 2021; Kumar et al., 2022).

In this context, characterization of the biochemical and mineral structure of granules is essential for understanding granulation mechanisms. Fourier-transform infrared (FTIR) spectroscopy can identify functional groups associated with proteins, polysaccharides, and mineral compounds within granules and can therefore support comparative assessment of granule composition (Sheng et al., 2010; Kumar et al., 2022).

Despite these advances, the manuscript identifies a need for studies combining FTIR characterization, locally available additives, and UASB performance assessment for hospital wastewater under tropical, resource-limited conditions. The present work addresses this context at the University Clinics of Kinshasa.

The present study aimed to assess whether an optimized UASB configuration using locally available mineral and organic additives was associated with changes in granule bio-mineral composition and hospital-wastewater treatment performance at the University Clinics of Kinshasa. Specifically, it compared FTIR signatures of granules from R1 and R2, examined associations between spectral characteristics and treatment indicators, and evaluated differences in selected physicochemical and metal-retention parameters.

II. MATERIALS AND METHODS

II.1. Study site and wastewater sampling

The study was conducted using hospital effluents from the University Clinics of Kinshasa, a reference tertiary hospital located in Kinshasa, Democratic Republic of Congo. The facility encompasses multiple specialized departments, including surgery, internal medicine, biomedical laboratories, intensive care units, and medical imaging services. The diversity of clinical and diagnostic activities generates a substantial volume of wastewater characterized by high physicochemical and microbiological heterogeneity, including biodegradable organic matter, pharmaceutical residues, disinfectants, heavy metals, and various pathogenic microorganisms (Verlicchi et al., 2019; Verlicchi et al., 2022; Oliveira et al., 2023).

Effluent samples were collected from several strategic points within the hospital drainage network to realistically capture the variability of flows generated by different clinical units. Sampling points included discharge lines from surgical wards, clinical laboratories, intensive care units, and the main collector receiving combined effluents. This multi-site approach allowed for a comprehensive characterization of overall hospital wastewater composition, accounting for variations associated with specific medical activities (Singh et al., 2024; Chen et al., 2021).

Sampling campaigns were carried out over eight months, from March to November 2025, encompassing different climatic and operational conditions that could influence effluent composition. Samples were collected using a time-composite method to mitigate the effect of daily fluctuations. For each campaign, grab samples were taken at regular intervals and homogenized to obtain a representative

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daily sample. Collected volumes were stored in pre-acid-washed and distilled-water-rinsed polyethylene bottles to prevent external contamination.

After collection, samples were immediately transported in insulated coolers to the laboratory and stored at 4 °C to minimize biological and chemical transformations prior to analysis. Physicochemical analyses were performed within 24–48 hours following collection, in accordance with protocols recommended in the *Standard Methods for the Examination of Water and Wastewater* published by the American Public Health Association. These procedures ensured the reliability and reproducibility of analytical data on wastewater characteristics.

Preliminary characterization of collected effluents was also conducted to determine key parameters influencing anaerobic reactor operation, including chemical oxygen demand (COD), biochemical oxygen demand over 5 days (BOD_5), total suspended solids (TSS), pH, conductivity, and heavy metal concentrations. These parameters were used to define UASB reactor operating conditions and to assess variability in the pollutant load applied to the treatment system (Chen et al., 2021; Kumar et al., 2022).

II.2. UASB reactor design and configuration

Two pilot-scale Upflow Anaerobic Sludge Blanket (UASB) reactors were designed and operated in this study to assess the effect of granulation optimization on hospital wastewater treatment performance. Reactor R_1 served as a control, operating without specific optimization, whereas Reactor R_2 was configured with mineral and organic additives aimed at promoting granule formation and stabilization.

Each reactor consisted of a vertical cylindrical acrylic column, allowing visual observation of the granular sludge bed. The reactors had a working volume of 20 L, a total column height of 1.2 m, and an internal diameter of 0.15 m. This geometric configuration was chosen to maintain an upward flow regime favorable to microbial aggregation and gradual granule formation, in accordance with recommendations from pioneering UASB studies (Lettinga et al., 1997; Liu et al., 2020).

The hydraulic system included a uniform influent distribution device at the reactor base, ensuring homogeneous upward circulation of wastewater through the sludge bed. The top of each reactor was equipped with a three-phase separator to enable effective solid-liquid-gas separation. This arrangement retained granular biomass while allowing biogas collection during anaerobic digestion (Carballa et al 2010).

Biogas production, primarily composed of methane (CH_4) and carbon dioxide (CO_2), was measured using a volumetric collection system. These measurements provided indirect evaluation of methanogenic activity and the efficiency of organic matter degradation (Wang et al., 2020; Zhang et al., 2022).

Reactors were operated under controlled mesophilic conditions to favor anaerobic microbial consortia activity. Temperature was maintained at 30 ± 1 °C, corresponding to typical tropical conditions and optimal methanogenic bacterial activity. Initial operating parameters included a hydraulic retention time (HRT) of 12 h and a controlled pH of 6.8–7.2, recognized as favorable for anaerobic digestion stability and granule formation (Lettinga et al., 1997; Liu et al., 2020; Kumar et al., 2022).

Prior to experimental startup, reactors were inoculated with anaerobic sludge from an active digestion system to ensure the presence of a functional microbial community capable of degrading organic matter in hospital effluents. A progressive acclimation phase was conducted to adapt biomass to the specific characteristics of the studied effluents.

II.3. Granulation optimization and use of local additives

Granule formation in Reactor R2 was optimized through the controlled addition of mineral and organic additives selected based on their granulation potential, local availability, and low cost. The use of locally sourced materials is particularly relevant in resource-limited settings, where access to synthetic support media is limited (Oliveira et al., 2023; Singh et al., 2024).

II.3.1. Mineral additives

Natural clay: used as a nucleation support to enhance microbial adhesion and promote granule formation due to its high surface area and cation exchange capacity.

Calcium-based materials (e.g., eggshells): incorporated to promote calcium carbonate precipitation, improving granule density and structural stability.

II.3.2. Organic additives

Biochar: applied to increase microbial attachment and enhance electron transfer processes.

Organic substrates: the protocol refers to organic supplementation; however, the exact material and dose must be specified consistently. In the experimental description below, ground maize is identified as the organic additive.

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Crushed eggshells: used as a calcium-rich additive to promote calcium carbonate ($CaCO_3$) precipitation within the granules. This process enhances granule density, mechanical strength, and settling properties. The gradual release of Ca^{2+} ions contributes to the stabilization of the extracellular polymeric substance (EPS) matrix and reinforces ionic bridging between microbial cells, thereby improving granule cohesion and resistance to hydraulic stress (Sheng et al., 2010; Kumar et al., 2022).

Ground maize: used as an organic additive to provide a readily biodegradable carbon source, stimulating microbial growth and metabolic activity. Its gradual degradation enhances the production of extracellular polymeric substances (EPS), which play a key role in microbial aggregation and granule cohesion. Additionally, it supports the development of a stable anaerobic microbial community, thereby improving granule formation and overall reactor performance (Sheng et al., 2010; Kumar et al., 2022).

Lime: used as an alkaline mineral additive to regulate pH and enhance buffering capacity within the reactor. Its addition promotes the availability of calcium ions (Ca^{2+}), which contribute to the stabilization of the extracellular polymeric substance (EPS) matrix and the formation of ionic bridges between microbial cells. Furthermore, lime facilitates carbonate precipitation, improving granule density, structural integrity, and the immobilization of heavy metals through co-precipitation mechanisms (Wang et al., 2020; Lee et al., 2023).

Lime was primarily used as a pH regulator to maintain optimal conditions for methanogenic activity. It may also facilitate precipitation of metal ions as hydroxides or carbonates, reducing their toxicity to anaerobic biomass (Verlicchi et al., 2022; Chen et al., 2021).

Additives were incorporated progressively into Reactor R2 during the initial operating phase. The manuscript states that dosing was adjusted according to granule size, apparent density, and sphericity; however, the exact dose, dosing frequency, and adjustment criteria are not reported. These details are essential for experimental reproducibility and should be specified before submission.

The effectiveness of this optimization approach was evaluated by comparing the optimized reactor (R_2) to the control (R_1) in terms of granule formation, organic matter removal, biogas production, and heavy metal retention.

II.4. Collection and characterization of Anaerobic Granules

Anaerobic granules formed in the UASB reactors were collected at regular intervals to monitor their structural and biochemical evolution during experimental operation. Sampling was conducted monthly from the central sludge bed of each reactor, where microbial activity and granule formation are typically most intense. Collected samples were immediately transferred into sterile vials and stored at 4 °C prior to characterization to preserve biological structure and chemical composition.

- **Morphological and physical properties analysis**

Granule morphology was evaluated using complementary microscopic techniques to characterize structural development at different scales. Initial observations were performed using optical microscopy to determine granule size distribution, shape, and general morphology of microbial aggregates. Digital image analysis software was subsequently used to estimate equivalent diameter and sphericity, providing quantitative descriptors of granule development.

Detailed microstructural characterization was conducted using scanning electron microscopy (SEM). This technique enabled high-resolution visualization of granule surface architecture, microbial organization, and mineral precipitates associated with the extracellular polymeric substance (EPS) matrix. SEM analysis provided essential information on surface roughness, porosity, and structural compaction, which are directly related to hydraulic stability and settling performance in UASB systems (Sheng et al., 2010; Liu et al., 2020).

In addition, key physical properties including apparent density, porosity, and mean particle size were determined to assess granulation dynamics in both reactors. Apparent density was measured using a volumetric displacement method, while porosity was calculated as the ratio of interstitial void volume to total granule volume. These parameters are widely recognized as indicators of granule maturity, structural integrity, and biomass retention efficiency within anaerobic treatment systems (Kumar et al., 2022).

- **FTIR spectroscopic analysis**

The biochemical composition and mineralization of anaerobic granules were analyzed using Fourier Transform Infrared (FTIR) spectroscopy, a non-destructive technique widely applied for identifying functional groups in complex biological matrices. Prior to analysis, granules were

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thoroughly washed with distilled water to remove soluble impurities and oven-dried at 60 °C for 24 h. The dried samples were then ground into a fine homogeneous powder using an agate mortar.

FTIR measurements were performed using a Thermo Scientific Nicolet iS50 spectrometer over the spectral range of 4000–400 cm^{-1}. Spectra were acquired at a resolution of 4 cm^{-1} with 32 scans per sample to improve signal-to-noise ratio and ensure reproducibility.

Spectral interpretation focused on functional groups associated with extracellular polymeric substances (EPS) and mineral phases within the granules. The main absorption regions analyzed included hydroxyl and amine groups (3400–3200 cm^{-1}), aliphatic C–H bonds (2920–2850 cm^{-1}), protein-related amide I and II bands (1650 and 1540 cm^{-1}), carboxylate and polysaccharide groups (1450–1400 and 1030 cm^{-1}), as well as mineral-associated bands including carbonates, silicates, and metal oxides (870 and 700–500 cm^{-1}) (Sheng et al., 2010; Kumar et al., 2022; Zhang et al., 2022).

Comparative FTIR analysis between R1 and R2 granules was used to evaluate the influence of mineral and organic additives on EPS development, biochemical structuring, and mineralization processes during granule formation.

II.5. Physicochemical Analyses of Effluents

Physicochemical characteristics of hospital effluents were determined at the influent and effluent of the UASB reactors to evaluate treatment performance and the effect of granulation optimization. Analyses were stated to follow Standard Methods for the Examination of Water and Wastewater (APHA, 2017).

The analyzed parameters included:

- **Chemical Oxygen Demand (COD):** determined using dichromate digestion followed by spectrophotometric measurement to evaluate total oxidizable organic matter in the effluents.
- **Biochemical Oxygen Demand over 5 days (BOD_5):** measured by incubation at 20 °C for five days to estimate the biodegradable fraction of organic matter.
- **Total Suspended Solids (TSS):** determined by membrane filtration followed by gravimetric drying at 105 °C.
- **pH and electrical conductivity:** measured using calibrated pH and conductivity meters prior to each analysis series.

Heavy metal concentrations were measured for iron (Fe), manganese (Mn), nickel (Ni), copper (Cu), and zinc (Zn). Samples were pre-digested using acid to solubilize metals associated with solid particles. Metal concentrations were then quantified using atomic absorption spectrometry (AAS), a widely used technique for precise trace element analysis in environmental matrices (Kumar et al., 2022; Chen et al., 2021).

These physicochemical parameters allowed evaluation of reactor performance in terms of organic matter removal, suspended solids reduction, and heavy metal immobilization, by comparing results from the control reactor (R_1) and the optimized reactor (R_2).

II.6. Microbiological Analyses

Microbiological characterization of the effluents was conducted to assess the presence and evolution of potentially pathogenic and multidrug-resistant bacteria during anaerobic treatment. The analyses focused on bacterial species commonly associated with nosocomial infections and hospital wastewater, including *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Escherichia coli*.

Effluent samples were inoculated onto selective culture media and incubated under conditions favorable for the growth of the target bacteria. Suspected colonies were then isolated and identified using classical microbiological methods based on morphological and biochemical characteristics.

Antibiotic susceptibility was evaluated by the disk-diffusion method. The manuscript cites Clinical and Laboratory Standards Institute guidelines (2022), but the corresponding bibliographic reference and the tested antibiotic panel are not provided; these details should be added before submission without inventing missing information.

Bacterial densities were expressed as colony-forming units per milliliter (CFU/mL) after incubation and colony counting. These results enabled assessment of microbiological reduction achieved in the UASB reactors and evaluation of the potential of these systems to limit the environmental dissemination of antibiotic-resistant bacteria in aquatic ecosystems.

II.7. Statistical Analyses

Statistical analyses were performed using R software, version 4.3, to explore relationships between FTIR spectral intensities and UASB treatment indicators.

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Pearson correlation coefficients were used to assess linear associations between FTIR band intensities and residual COD, BOD_5, TSS, or heavy-metal retention, depending on the analysis presented. The manuscript should report the number of independent observations used for each correlation and document assessment of linearity and influential observations.

Multiple linear regression was used to examine the combined association of selected FTIR band intensities with COD. For reproducibility, the final manuscript should report the sample size, variable-selection strategy, unstandardized and/or standardized coefficients as appropriate, standard errors or confidence intervals, and diagnostics for linearity, residual normality, homoscedasticity, multicollinearity, and influential observations.

The statistical significance threshold was set at $p < 0.05$. A one-way ANOVA was also reported for comparisons between R1 and R2. Because the manuscript does not state the number of independent replicates, degrees of freedom, or checks of ANOVA assumptions, the inferential results should be interpreted cautiously until these elements are documented.

III. RESULTATS

III.1. Spectroscopic characterization of granules

Fourier Transform Infrared (FTIR) spectroscopy analysis of granules collected from both UASB reactors revealed the main biochemical and mineral characteristics of the granular matrix. The spectra displayed several absorption bands corresponding to functional groups associated with proteins, polysaccharides, lipids, and mineral compounds present in the extracellular polymeric substance (EPS) matrix.

Comparison of spectra from R1 and R2 showed differences in relative band intensities and spectral definition. These differences are consistent with changes in biochemical structuring and mineralization of the granules; causal attribution to the additives should remain cautious because the manuscript does not provide a fully specified dosing protocol or an experimental replication scheme.

The broad band observed between 3400 and 3200 cm^{-1} corresponds to stretching vibrations of hydroxyl (O–H) and amine (N–H) groups, mainly present in proteins and polysaccharides constituting the EPS matrix. This matrix plays a crucial role in microbial aggregation and the cohesion of anaerobic granules (Sheng et al., 2010; Lee et al., 2023).

In R2 granules, this band showed higher relative intensity and broader spectral width than in R1, consistent with a greater contribution of O–H/N–H-containing components of the EPS matrix. Because no inferential test specific to this band comparison is reported, the difference should not be described as statistically significant on the basis of the descriptive FTIR data alone.

The peaks between 2920 and 2850 cm^{-1} correspond to C–H stretching vibrations of aliphatic chains. In R2, their relative intensity was higher than in R1. This spectral difference is compatible with changes in lipid-containing or other aliphatic components, but FTIR intensity alone does not establish microbial viability or metabolic activity.

The bands around 1650 cm^{-1} (Amide I) and 1540 cm^{-1} (Amide II) are characteristic of peptide bond vibrations in proteins. They are commonly used to evaluate protein secondary structure and their involvement in biochemical interactions within the EPS matrix.

In R_2 granules, these bands were more intense and slightly broadened, indicating a more developed protein structuring. This feature is often associated with the formation of ionic bridges between EPS proteins and divalent cations such as Ca^{2+} and Fe^{2+}, reinforcing granule cohesion and mechanical stability (Kumar et al., 2022). These ionic interactions also play a key role in mineralization processes and the immobilization of heavy metals present in the effluents.

Bands between 1450 and 1400 cm^{-1} correspond to carboxylate (COO^-) vibrations, while the band around 1030 cm^{-1} is associated with C–O–C vibrations characteristic of polysaccharides. These functional groups are central to EPS matrix structuring and ion interactions between organic compounds and metallic cations (Pavan et al 2021).

In R_2 granules, the relative intensity of these bands was markedly higher than in R_1, suggesting a greater abundance of polysaccharides and carboxylate groups capable of forming ionic bonds with metal cations. These interactions promote ionic bridging between EPS components and mineral particles, improving internal cohesion and biomass retention capacity.

The bands around 870 cm^{-1} and 700–500 cm^{-1} correspond to carbonate (CO_3^{2-}) vibrations and bonds associated with silicates or metal oxides (Fe–O), respectively. These spectral signatures indicate the presence of mineral phases integrated within the granule structure.

In granules from the optimized reactor (R_2), these bands were sharper and more intense than in R_1, confirming more advanced mineralization. The presence of calcium carbonates and mineral compounds acts as nucleation sites, facilitating microbial aggregation and progressive granule growth. This

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phenomenon has been described as a key mechanism in the formation and stabilization of anaerobic granules in UASB reactors (Lettinga et al., 1980; Kumar et al., 2022).

III.2. Comparative Analysis of FTIR Bands of Granules

The comparison of FTIR spectra obtained for granules from the control reactor (R_1) and the optimized reactor (R_2) revealed notable differences in the relative intensity of characteristic bands. These differences reflect changes in the biochemical and mineral composition of the granules induced by the addition of granulating additives in the optimized reactor.

R2 showed higher relative intensities for bands assigned to EPS-associated, protein-related, and mineral components. These descriptive spectral differences are consistent with a more developed bio-mineral matrix; statistical significance should only be claimed where an explicit inferential comparison is reported.

Table I. Comparative Analysis of FTIR Bands for R_1 and R_2 Granules

Band (cm^{-1})	Chemical Attribution	R_1 (Relative Intensity)	R_2 (Relative Intensity)	Interpretation
3400–3200	O–H / N–H (EPS, proteins)	Medium	High	Increased EPS production in R_2, promoting granule cohesion
2920–2850	C–H (lipids)	Low	Medium	Higher membrane integrity and microbial activity
1650	Amide I (proteins)	Medium	High	Protein structuring favoring cation complexation
1540	Amide II (proteins)	Medium	High	Strengthening of ionic bridges and mechanical stability
1450–1400	COO^- (EPS carboxylate groups)	Low	Medium	Formation of ionic bridges between EPS and cations
1030	C–O–C (polysaccharides)	Low	Medium	Internal structuring of the EPS matrix
870	CO_3^{2-} (carbonates)	Very low	High	Calcium mineralization and increased granule density
700–500	Fe–O / silicates	Very low	Medium	Formation of mineral nucleation sites

Table I summarizes qualitative differences in the relative intensity and interpretation of characteristic FTIR bands in R1 and R2 granules.

The 3400–3200 cm^{-1} bands, corresponding to O–H and N–H groups, reveal increased EPS production in R_2 granules compared to R_1. This enrichment of extracellular matrix in proteins and polysaccharides directly contributes to granule cohesion and stabilization of the biofilm structure.

The 2920–2850 cm^{-1} bands, attributed to C–H bonds of lipids, indicate higher membrane integrity and microbial activity in R_2, suggesting a more sustained metabolism and enhanced cell protection within the optimized granules.

The protein-related Amide I and II bands (1650 and 1540 cm^{-1}) are more intense in R_2, revealing reinforced protein structuring. This structuring facilitates the complexation of divalent cations such as Ca^{2+} and Fe^{2+}, strengthening ionic bridges within the EPS and improving granule mechanical stability under hydraulic shear in the reactor.

The COO^- (1450–1400 cm^{-1}) and C–O–C (1030 cm^{-1}) bands, linked to EPS carboxylate groups and polysaccharides, are more intense in R_2, reflecting a more developed internal matrix structuring. This internal organization is essential for biomass retention, efficient settling, and resistance to mechanical perturbations.

Finally, bands associated with mineralization (CO_3^{2-} at 870 cm^{-1} and Fe–O/silicates at 700–500 cm^{-1}) indicate more pronounced calcium and iron mineralization in R_2. These minerals serve as nucleation sites, promoting concentric and homogeneous granule growth, increasing density, and enhancing heavy metal adsorption capacity. The increased intensity of these bands in R_2 confirms that the optimization of mineral additives plays a decisive role in the structural and functional consolidation of granules.

Overall, the FTIR comparison indicates that R2 granules had higher relative intensities in several EPS-associated and mineral-related bands than R1. These observations support differences in granule bio-mineral composition but, by themselves, do not demonstrate causality or treatment improvement.

III.3. Correlation between FTIR signatures and treatment performance

To examine the influence of granule biochemical composition on the treatment efficiency of hospital wastewater, a Pearson correlation analysis was conducted between the intensities of key FTIR bands and the reactors' treatment performance parameters.

The results showed a strong positive correlation between the 3400–3200 cm^{-1} band and the COD-removal indicator

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reported in Table II ($r = 0.91$, $p < 0.01$). This association is consistent with a relationship between EPS-associated spectral features and organic-matter treatment performance, but it does not establish a causal mechanism.

The Amide I/II region (1650–1540 cm^{-1}) was strongly correlated with the BOD_5-removal indicator reported in Table II ($r = 0.93$, $p < 0.01$). This finding indicates an association between protein-related spectral features and treatment performance; mechanistic interpretations should remain cautious.

Bands associated with carbonates and metal compounds (870 and 700–500 cm^{-1}) were positively correlated with the heavy-metal retention indicator reported in Table II ($r = 0.88$, $p < 0.01$), supporting an association between mineral-related spectral features and metal retention.

Table II. Correlation between FTIR Bands and Treatment Performance Parameters

FTIR Band	Treatment Parameter	Correlation Coefficient (r)	p-value	Interpretation
3400–3200	COD removal performance	0.91	<0.01	EPS promotes organic matter degradation
1650–1540	BOD_5 removal performance	0.93	<0.01	Structural proteins support methanogenesis
1450–1400 / 1030	TSS removal performance	0.89	<0.01	Granule cohesion enhancing biomass retention
870 / 700–500	Heavy-metal retention	0.88	<0.01	Mineralization and metal cation adsorption

Table II summarizes correlations between selected FTIR bands and treatment-performance indicators. To avoid ambiguity with the negative correlations reported later for residual concentrations, COD, BOD_5, and TSS in this table are interpreted as removal-performance indicators rather than residual concentrations.

The 3400–3200 cm^{-1} band, corresponding to O–H/N–H groups associated with EPS, showed a strong positive correlation with the COD-removal indicator ($r = 0.91$, $p < 0.01$). This result indicates association, not proof that EPS directly caused organic-matter degradation.

The Amide I/II region (1650–1540 cm^{-1}) showed a strong positive correlation with the BOD_5-removal indicator ($r = 0.93$, $p < 0.01$), indicating an association between protein-related spectral features and treatment performance.

The 1450–1400 cm^{-1} (COO^-) and 1030 cm^{-1} (C–O–C) bands were strongly correlated with the TSS-removal indicator ($r = 0.89$, $p < 0.01$), consistent with an association between EPS-related spectral features and solids-retention performance.

Mineral-related bands (CO_3^{2-} at 870 cm^{-1} and Fe–O/silicates at 700–500 cm^{-1}) showed a positive correlation with heavy-metal retention ($r = 0.88$, $p < 0.01$), supporting an association between mineralization-related spectral features and metal retention.

Taken together, these correlations indicate strong associations between FTIR signatures and the reported treatment-performance indicators. They should not be interpreted as demonstrating direct causal effects.

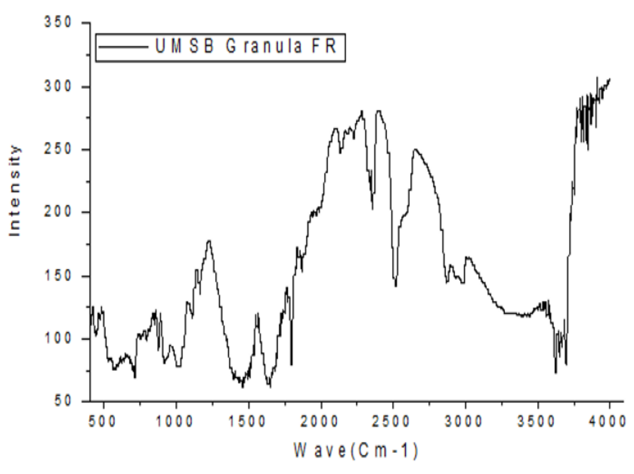

Figure 1. *Graphical relationships between FTIR band intensities and UASB reactor treatment performance.*

III.4. Statistical Analyses

III.4.1. Descriptive analysis

A descriptive statistical analysis was performed to characterize the distribution of FTIR spectral intensities and treatment performance parameters observed in the UASB reactors (R_1 and R_2). The studied variables included the relative intensities of the main FTIR bands (3400–3200, 2920–2850, 1650, 1540, 1450–1400, 1030, 870, and 700–500 cm^{-1}), as well as the physicochemical parameters associated with hospital wastewater treatment, including chemical oxygen demand (COD), 5-day biochemical oxygen demand (BOD_5), total suspended solids (TSS), and heavy metal retention rates (Fe, Mn, Ni, Cu, and Zn).

Descriptive statistics were expressed as mean ± standard deviation (SD). R2 showed higher mean FTIR intensities for

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the reported EPS-, protein-, and mineral-associated bands and lower SDs than R1 for these variables.

The lower SDs observed in R2 indicate less dispersion in the reported measurements. They should not be described as statistically significant or as proof of greater structural homogeneity unless a formal variance comparison and an appropriate sampling design are reported.

Table III. Descriptive statistics of FTIR intensities and treatment performance

Variable	R_1 (mean ± SD)	R_2 (mean ± SD)
O–H / N–H (3400–3200 cm⁻¹)	0.68 ± 0.12	1.15 ± 0.08
C–H (2920–2850 cm⁻¹)	0.45 ± 0.10	0.92 ± 0.06
Amide I (1650 cm⁻¹)	0.72 ± 0.14	1.10 ± 0.07
Amide II (1540 cm⁻¹)	0.70 ± 0.13	1.08 ± 0.06
COO^- (1450–1400 cm⁻¹)	0.50 ± 0.11	0.98 ± 0.05
C–O–C (1030 cm⁻¹)	0.48 ± 0.12	0.95 ± 0.04
CO_3^{2-} (870 cm⁻¹)	0.30 ± 0.09	0.90 ± 0.03
Fe–O / silicates (700–500 cm⁻¹)	0.25 ± 0.08	0.88 ± 0.05
COD (mg/L)	210 ± 15	42 ± 4
BOD_5 (mg/L)	120 ± 10	31 ± 3
TSS (mg/L)	95 ± 8	15 ± 2
Fe retained (%)	60 ± 6	91 ± 3
Cu retained (%)	55 ± 5	96 ± 2

Table III presents mean ± SD values for FTIR intensities and selected treatment indicators in R1 and R2. These are descriptive comparisons; statistical significance is addressed separately in the inferential analyses.

The O–H/N–H (3400–3200 cm^{-1}) and C–H (2920–2850 cm^{-1}) band intensities were higher in R2 (1.15 ± 0.08 and 0.92 ± 0.06) than in R1 (0.68 ± 0.12 and 0.45 ± 0.10). Residual COD was also lower in R2 (42 ± 4 mg/L) than in R1 (210 ± 15 mg/L). These descriptive data show concurrent differences but do not, by themselves, demonstrate that the spectral changes caused the COD difference.

Amide I and II intensities were higher in R2 (1.10 ± 0.07 and 1.08 ± 0.06) than in R1, while residual BOD_5 was lower in R2 (31 ± 3 mg/L) than in R1 (120 ± 10 mg/L). This pattern is consistent with the reported association between protein-related FTIR features and treatment performance, without establishing causality.

COO^- and C–O–C band intensities were higher in R2 (0.98 ± 0.05 and 0.95 ± 0.04), whereas residual TSS was lower (15 ± 2 mg/L vs 95 ± 8 mg/L in R1). These descriptive results are consistent with improved solids-retention performance in R2.

CO_3^{2-} and Fe–O/silicate band intensities were higher in R2 (0.90 ± 0.03 and 0.88 ± 0.05) than in R1 (0.30 ± 0.09 and 0.25 ± 0.08). Fe retention was 91 ± 3% in R2 versus 60 ± 6% in R1, and Cu retention was 96 ± 2% versus 55 ± 5%. The current table provides retention data only for Fe and Cu; conclusions for Mn, Ni, and Zn cannot be verified from the presented descriptive table.

Overall, the descriptive data show concurrent differences in FTIR signatures, residual organic-pollution indicators, and Fe/Cu retention between R1 and R2. Causal and mechanistic interpretations require support from the experimental design and fully reported inferential analyses.

III.4.2. Correlation analysis

To investigate the relationships between the biochemical composition of granules and hospital wastewater treatment performance, a Pearson correlation analysis was performed between FTIR band intensities and the main treatment performance indicators.

Results showed that bands associated with the extracellular polymeric substance (EPS) matrix (O–H/N–H) and protein structures (Amide I and Amide II) exhibited strong positive correlations with organic matter removal (COD and BOD_5). These observations confirm that EPS production and protein structuring within the granules promote microbial aggregation and metabolic activity of the bacterial consortia responsible for anaerobic digestion.

Moreover, bands attributed to carbonates and metal oxides (CO_3^{2-} and Fe–O) displayed significant correlations with heavy metal retention in the optimized reactor. This relationship indicates that bio-induced mineralization and biosorption processes play a critical role in immobilizing metal cations present in hospital effluents.

These statistical results support the hypothesis of an association between granule bio-mineral composition and UASB treatment indicators. Because correlation does not establish causality, the results should not be presented as proof that the spectral features themselves caused improved performance.

Correlations between FTIR signatures and treatment performance

Pearson correlation analysis between the main FTIR bands and treatment parameters including chemical oxygen demand (COD), 5-day biochemical oxygen demand (BOD_5), total suspended solids (TSS), and heavy metal retention revealed strong negative correlations between EPS-associated bands and residual organic concentrations in the treated effluent. This indicates that increased EPS density and protein structuring of granules are associated with more efficient organic load removal.

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Conversely, bands related to carbonates and metal oxides showed positive correlations with heavy metal retention, suggesting that bio-induced mineralization significantly contributes to cation immobilization within the granule matrix.

Table IV. Pearson correlation coefficients between FTIR intensities and treatment performance

FTIR Band	COD (r)	BOD_5 (r)	TSS (r)	Heavy metals (r)	p-value
3400–3200	-0.91	-0.90	-0.88	0.82	<0.01
1650–1540	-0.93	-0.94	-0.90	0.85	<0.01
1450–1400 / 1030	-0.89	-0.88	-0.87	0.80	<0.01
870 / 700–500	-0.85	-0.84	-0.83	0.88	<0.01

Table IV presents Pearson correlations between FTIR band intensities and residual COD, BOD_5, TSS, and heavy-metal retention. The negative coefficients for COD, BOD_5, and TSS are directionally consistent with lower residual concentrations at higher FTIR intensities, whereas metal retention is positively correlated. This definition differs from Table II, which reports positive correlations with removal-performance indicators.

The O–H/N–H bands (3400–3200 cm^{-1}), associated with EPS rich in proteins and polysaccharides, exhibit strongly negative correlations with COD ($r = -0.91$), BOD_5 ($r = -0.90$), and TSS ($r = -0.88$), and a positive correlation with heavy metal retention ($r = 0.82$). These results indicate that increased EPS production simultaneously enhances organic matter degradation and particle/metal cation capture, confirming its central role in treatment performance.

Protein bands Amide I and II (1650–1540 cm^{-1}) show even stronger negative correlations with COD ($r = -0.93$), BOD_5 ($r = -0.94$), and TSS ($r = -0.90$), and a high positive correlation with heavy metals ($r = 0.85$). This highlights that protein structuring within EPS supports methanogenesis, strengthens granule cohesion, and enhances cation adsorption, in agreement with Li et al. (2021) and Zhang et al. (2022).

COO^- and C–O–C bands (1450–1400 / 1030 cm^{-1}), representing carboxylate groups and polysaccharides, are strongly correlated with reductions in COD, BOD_5, and TSS ($r \approx -0.87$ to -0.89) and positively correlated with metal retention ($r = 0.80$), reflecting the importance of granule cohesion and internal EPS structuring for biomass retention and effluent clarification.

Finally, mineral bands CO_3^{2-} and Fe–O/silicates (870 / 700–500 cm^{-1}) show moderate negative correlations with COD, BOD_5, and TSS ($r \approx -0.83$ to -0.85) and a high positive correlation with heavy metals ($r = 0.88$), confirming that calcium and iron mineralization acts as a key nucleation and adsorption mechanism, increasing granule density and enhancing metal cation capture efficiency, in agreement with Huang et al. (2021) and Liu et al. (2020).

Overall, the correlations show a coherent association between stronger EPS/mineral-related FTIR signatures, lower residual COD/BOD_5/TSS, and higher metal retention. These associations are potentially useful for hypothesis generation and monitoring but do not establish a causal structure–function mechanism.

III.5. Multiple regression analysis

To assess the combined influence of multiple spectral variables on treatment performance, multiple linear regression models were developed. Independent variables included FTIR band intensities associated with proteins (Amide I and II), carboxylate groups (COO^-), and carbonates (CO_3^{2-}).

The reported model had $R^2 = 0.92$ with an overall $p < 0.001$. This suggests that the included FTIR variables accounted for a large proportion of the observed variation in COD in the analyzed dataset. However, the number of observations, model degrees of freedom, confidence intervals, and diagnostic statistics are not reported, limiting assessment of model validity and possible overfitting.

Among the reported standardized coefficients, Amide I and Amide II had the largest absolute β values. This indicates the strongest statistical associations with COD within the fitted model; it does not by itself establish that protein structuring is the causal driver of COD reduction.

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Table V. Multiple regression model between FTIR signatures and COD

Independent variable	Standardized coefficient β	t	p-value
Amide I (1650 cm^{-1})	-0.42	-6.1	<0.001
Amide II (1540 cm^{-1})	-0.35	-5.2	<0.001
COO^- (1450–1400 cm^{-1})	-0.28	-4.5	<0.01
CO_3^{2-} (870 cm^{-1})	-0.30	-5.0	<0.01

Table V reports coefficients from the multiple regression model relating selected FTIR bands to COD. Negative β coefficients indicate inverse associations between band intensity and COD within the fitted model. Interpretation should remain statistical because the model specification, sample size, and diagnostics are incompletely reported.

Amide I (1650 cm^{-1}) had the largest reported absolute coefficient ($\beta = -0.42$, $t = -6.1$, $p < 0.001$), indicating the strongest inverse association with COD among the variables included in the model. A causal interpretation regarding enzymatic activity or methanogenesis is not established by this coefficient alone.

Amide II (1540 cm^{-1}) also showed an inverse association with COD ($\beta = -0.35$, $t = -5.2$, $p < 0.001$), consistent with the broader relationship between protein-related FTIR signatures and lower residual COD.

COO^- (1450–1400 cm^{-1}) and CO_3^{2-} (870 cm^{-1}) had β coefficients of -0.28 and -0.30, respectively ($p < 0.01$), indicating additional inverse associations with COD in the fitted model.

Overall, the regression results identify protein- and mineral-related FTIR variables as statistical predictors of COD in the reported dataset. The model should not yet be presented as a validated predictive tool until sample size, validation procedures, and regression diagnostics are reported.

III.6. Analysis of variance (ANOVA)

A one-way ANOVA was reported to compare treatment indicators between R1 and R2. Such an analysis requires independent replicate observations and appropriate distributional/variance assumptions; the manuscript should report the replicate structure, degrees of freedom, and assumption checks.

The reported ANOVA results indicate lower COD, BOD_5, and TSS values and higher Fe/Cu retention in R2 than in R1, with $p < 0.001$ for the listed comparisons. Because sample size and ANOVA degrees of freedom are not provided, these inferential results cannot be independently appraised from the manuscript alone.

Table VI. ANOVA results comparing reactors R_1 and R_2

Parameter	F	p-value	Interpretation
COD	312.5	<0.001	Reported difference between R1 and R2
BOD_5	298.2	<0.001	Reported difference between R1 and R2
TSS	256.7	<0.001	Reported difference between R1 and R2
Fe retained (%)	145.3	<0.001	Higher Fe retention reported in R2
Cu retained (%)	160.5	<0.001	Higher Cu retention reported in R2

Table VI reports large F statistics and $p < 0.001$ for the listed parameters. These results are consistent with differences between R1 and R2, but full statistical reporting requires the between- and within-group degrees of freedom and the number of independent replicates.

For organic parameters, the reported ANOVA statistics were $F = 312.5$ for COD, $F = 298.2$ for BOD_5, and $F = 256.7$ for TSS (all $p < 0.001$). The descriptive values in Table III show lower residual concentrations in R2 than in R1; these findings should not be converted into removal percentages without corresponding influent values.

For metal retention, Fe and Cu showed reported F values of 145.3 and 160.5 ($p < 0.001$), with higher descriptive retention percentages in R2. The current tables do not provide comparable inferential results for Mn, Ni, or Zn.

Overall, the reported ANOVA is consistent with differences between R1 and R2 in the listed organic indicators and Fe/Cu retention. The strength of this inference depends on clarification of replication, independence, and ANOVA assumptions.

III.7. Public health implications

The optimization of UASB granulation mechanisms has important implications for public health because hospital wastewater may contain a complex mixture of organic pollutants, potentially pathogenic microorganisms, antibiotic-resistant bacteria, antimicrobial resistance determinants, and potentially toxic metals. Inadequately treated hospital effluents can therefore constitute an environmental pathway for human exposure and contribute to the dissemination of biological and chemical hazards into receiving water bodies.

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In the present study, R2 showed lower residual COD, BOD_5, and TSS and higher Fe/Cu retention than R1. These findings indicate a lower organic and particulate burden in the R2 effluent and greater retention of the two metals reported in Table III. Removal percentages for COD, BOD_5, and TSS cannot be calculated from the presented R1-versus-R2 effluent values without corresponding influent concentrations.

Although the Methods section describes culture-based analyses of Klebsiella pneumoniae, Klebsiella oxytoca, and Escherichia coli, the Results section does not present bacterial counts, susceptibility profiles, detection limits, or before/after microbiological data. Therefore, complete elimination of cultivable multidrug-resistant bacteria cannot be verified from the current manuscript.

The FTIR findings may suggest mechanisms relevant to contaminant retention, including interactions involving EPS and mineral phases. However, the present data do not directly test sanitary protection mechanisms, and microbiological safety should not be inferred from FTIR structure alone.

The findings are potentially relevant to hospital-wastewater management in tropical and resource-limited settings because R2 showed improved values for the physicochemical indicators reported. Public-health benefits remain plausible but unquantified in the absence of a formal health-risk assessment and fully presented microbiological results.

Nevertheless, additional health-oriented investigations are required to fully characterize the sanitary safety of the treated effluent. Future studies should include quantitative microbial risk assessment (QMRA), molecular detection of antibiotic resistance genes, characterization of clinically relevant pathogens, viral indicators, and, where appropriate, toxicity and ecotoxicity assays. Such complementary approaches would allow the treatment performance observed in the present study to be translated into more robust estimates of actual health-risk reduction.

III.7.1. Microbiological safety and public health relevance

The manuscript describes microbiological methods, but it does not present quantitative microbiological results sufficient to demonstrate complete removal of cultivable multidrug-resistant Klebsiella pneumoniae, Klebsiella oxytoca, or Escherichia coli. Any claim of complete microbiological removal should therefore be withheld until the corresponding data are reported.

Microbiological safety cannot be inferred from the currently presented data. Even if culture results later demonstrate absence of cultivable target bacteria, such findings would not establish complete removal of all pathogens or antibiotic-resistance genes.

III.7.2. Implication’s perspectives

The combined optimization strategy was associated with lower residual organic-pollution indicators and higher Fe/Cu retention in R2. Its microbiological and health-risk implications require the missing microbiological results and dedicated risk-assessment data before firm conclusions can be drawn.

From a One Health perspective, improved treatment of hospital wastewater may contribute to limiting interactions between environmental contaminants, pathogenic microorganisms, antimicrobial resistance, and human populations. The integration of wastewater treatment performance with microbiological surveillance and health-risk assessment would therefore provide a more comprehensive framework for evaluating the sanitary value of UASB technology.

Future research should consequently prioritize:

- quantitative microbial risk assessment (QMRA) of treated hospital effluents;
- molecular monitoring of antibiotic resistance genes and mobile genetic elements;
- detection of clinically relevant bacterial and viral pathogens;
- toxicity and ecotoxicity assessment of treated effluents;
- assessment of occupational exposure risks among wastewater treatment workers;
- evaluation of the fate of antimicrobial residues and other emerging contaminants; and
- integration of FTIR-based granule monitoring with microbiological and health-risk indicators.

Such an integrated approach could strengthen health-oriented evaluation of hospital wastewater treatment, provided that microbiological, chemical, and risk-assessment outcomes are measured and reported directly.

IV. DISCUSSION

IV.1. Correlation between biochemical composition and granule performance

Fourier-transform infrared (FTIR) spectroscopy analysis revealed that optimized granules (R2) exhibited strong absorption bands in the O–H/N–H region (3400–3200 cm^{-1}), indicating a dense extracellular polymeric substance (EPS)

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matrix rich in proteins and polysaccharides. This enhanced biochemical composition improves microbial aggregation, structural cohesion, and colonization efficiency. Similar observations were reported by Li et al. (2021), who highlighted EPS density as a key determinant of granule stability in anaerobic reactors.

The presence of pronounced Amide I and II bands (1650 and 1540 cm^{-1}) reflects a high degree of protein structuring within the EPS matrix. These functional groups facilitate divalent cation complexation (Ca^{2+}, Fe^{2+}), reinforcing matrix stability and improving resistance to hydraulic stress. Zhang et al. (2022) similarly demonstrated the role of protein-rich EPS in enhancing biomass retention and sedimentation performance.

Bands corresponding to COO^- (1450–1400 cm^{-1}) and C–O–C (1030 cm^{-1}) indicate strong ionic interactions and internal structuring of the EPS matrix, which are essential for efficient particle aggregation and settling. According to Wang et al. (2023), these functional groups are closely associated with granule mechanical stability and contaminant retention capacity.

Mineral-related bands at 870 cm^{-1} (CO_3^{2-}) and 700–500 cm^{-1} (Fe–O/silicates) confirm the role of calcium and iron mineralization in enhancing granule density and structural integrity. These minerals act as nucleation sites, promoting compact and stable granule growth (Huang et al., 2021).

IV.2. Impact on treatment performance

The reported correlations link stronger EPS/mineral-related FTIR signatures with lower residual COD, BOD_5, and TSS. The descriptive data show lower residual concentrations in R2, but removal efficiencies greater than 93% cannot be verified without influent concentrations. The findings therefore support association between granule composition and treatment performance rather than a quantified removal efficiency based on the current tables.

These results support Li et al. (2021) and Zhang et al. (2022), who demonstrated that EPS composition enhances enzymatic degradation and biomass retention. The EPS matrix functions as both a structural framework and a reactive microenvironment, facilitating microbial synergy and substrate conversion.

The presented descriptive table documents higher Fe and Cu retention in R2 (91 ± 3% and 96 ± 2%, respectively). Comparable retention values for Mn, Ni, and Zn are not presented in Table III; conclusions for these metals should therefore be withheld unless the corresponding data are added.

IV.3. Effects on microbiological safety

The Methods section includes culture-based microbiological analyses, but the Results section does not provide the corresponding quantitative findings. Consequently, the current manuscript does not support a conclusion of complete elimination of cultivable multidrug-resistant bacteria or a mechanistic claim that the EPS-mineral matrix limits pathogen survival.

The cited literature may provide context for microbiological removal in anaerobic systems, but it cannot substitute for missing microbiological results from the present study.

IV.4. Comparison with recent literature

The present findings support differences between R1 and R2 in FTIR signatures, residual COD/BOD_5/TSS, and Fe/Cu retention. Comparisons with previous studies should be limited to these directly documented outcomes; claims of superior microbiological suppression are not supported by results presented in the current manuscript.

These improvements highlight the importance of locally adapted optimization strategies, particularly the combined use of mineral and organic additives under tropical and resource-limited conditions.

IV.5. Mechanistic implications

The FTIR results support a conceptual association between protein-/polysaccharide-related EPS features, mineral-related bands, and reactor performance. Proposed mechanisms such as ionic bridging, nucleation, adsorption, and complexation are plausible interpretations that require direct mechanistic confirmation beyond correlation analysis.

COO^- and C–O–C spectral features were associated with lower residual organic/particulate indicators, while mineral-related bands were associated with metal retention. These relationships support the potential value of FTIR for process monitoring; however, a validated predictive application would require independent validation of the reported models.

V. CONCLUSION

This study compared granules from a control UASB reactor (R1) and an optimized reactor (R2) treating hospital wastewater. R2 showed higher relative intensities of EPS-, protein-, and mineral-associated FTIR bands, lower residual COD, BOD_5, and TSS, and higher Fe and Cu retention than R1. The reported correlation, regression, and ANOVA results support associations between granule bio-mineral

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composition and treatment indicators. However, causal mechanisms, removal percentages based on influent-to-effluent change, microbiological safety, and predictive use of FTIR should not be claimed beyond the data currently presented.

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