

Heavy metal removal from hospital effluents at the University Clinics of Kinshasa using a UASB reactor: A comparative analysis under optimised and non-optimised operational conditions

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ABSTRACT

Introduction

Hospital effluents often contain heavy metals that pose significant environmental and public health risks, particularly in settings with limited access to advanced wastewater treatment technologies. Upflow Anaerobic Sludge Blanket (UASB) reactors represent a low-cost alternative; however, pilot-scale data on heavy metal removal remain scarce.

Purpose

This study evaluated the performance of a pilot-scale UASB reactor for heavy metal removal under optimised and non-optimised operational conditions and identified key factors influencing treatment efficiency.

Methods

A Plackett-Burman experimental design was used to screen critical operational parameters. The UASB reactor was operated for 12 weeks using real hospital effluent collected from the University Clinics of Kinshasa, with 36 samples analysed per monitoring point. The optimised reactor (P14) incorporated natural additives, including clay, eggshells, maize, and lime. Heavy metal concentrations were determined using atomic absorption spectrophotometry (AAS), with quality assurance and quality control procedures, calibration standards, and analytical blanks applied throughout.

Results

Under optimised conditions, substantial reductions were observed in chemical oxygen demand (COD; $620 \rightarrow 150 \text{ mg/L}$) and heavy metal concentrations: iron ($1.20 \rightarrow 0.45 \text{ mg/L}$), copper ($0.85 \rightarrow 0.30 \text{ mg/L}$), and zinc ($0.60 \rightarrow 0.22 \text{ mg/L}$), corresponding to removal efficiencies of approximately 60–70%. Nickel and manganese concentrations also decreased but remained above World Health Organization guideline limits. Reactor performance remained stable throughout the 12-week operational period.

Conclusion

Pilot-scale UASB reactors supplemented with natural additives can significantly enhance heavy metal removal from hospital effluents. Further research is required to assess long-term operational stability, heavy metal fate, sludge management, and scalability prior to full-scale implementation.

INTRODUCTION

Hospital effluents represent a major environmental concern due to the presence of pathogens, pharmaceutical residues, and, in particular, heavy metals such as Pb, Cd, Cu, and Zn, which are toxic, persistent, and bioaccumulative (Verlicchi et al., 2010; Tchounwou et al., 2012). Inadequate treatment of these effluents promotes metal accumulation in aquatic ecosystems, leading to long-term ecological and human health risks (Khan et al., 2024).

Conventional wastewater treatment systems, especially activated sludge processes, generally exhibit limited efficiency for heavy metal removal and often require costly physicochemical post-treatments that generate secondary sludge (Fu & Wang, 2011). This limitation has increased interest in anaerobic technologies as more sustainable treatment alternatives.

Upflow Anaerobic Sludge Blanket (UASB) reactors are widely applied for the treatment of high-strength wastewaters and can retain heavy metals through sulfide precipitation, adsorption onto sludge, and interactions with extracellular polymeric substances (EPS) (Lettinga et al., 1991; Omil et al., 1997). These mechanisms are strongly influenced by operational parameters such as pH, temperature, hydraulic retention time, and influent metal concentrations (Chen et al., 2008; Mohan et al., 2007).

Experimental design tools, including the Plackett–Burman method, enable the identification of critical operational factors affecting reactor performance (Plackett & Burman, 1946). In this study, optimised and non-optimised UASB configurations were compared for heavy metal removal from hospital wastewater.

Natural low-cost materials, including clay, crushed eggshells, and maize, were incorporated into the optimised reactor to enhance sludge performance and metal retention. Eggshells act as calcium carbonate buffers that promote metal precipitation, while clay provides a high cation exchange capacity favourable for metal adsorption (Chubar et al., 2004; Mohan & Pittman, 2006).

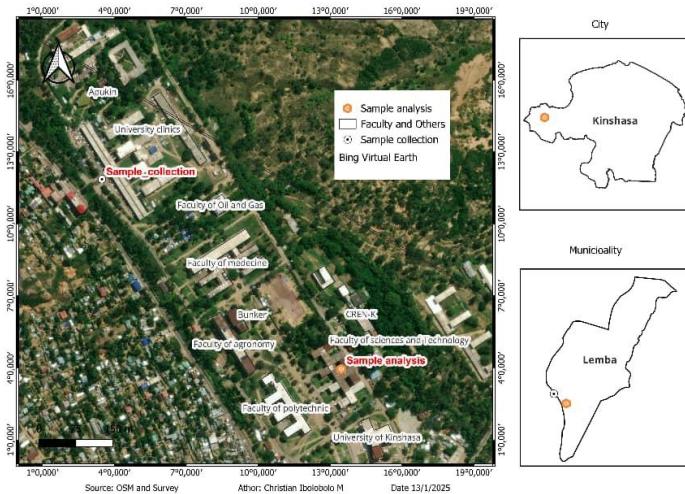
The objective of this study was to evaluate the contribution of optimised UASB operation combined with natural materials to heavy metal removal, highlighting its potential as a low-cost treatment option for hospital effluents.

METHODS

Study Site: University Clinics of Kinshasa (CUK)

Map 1:

Geolocation of the University Clinics of Kinshasa (sampling site) and the Faculty of Science and Technology (experimental site).



Hospital effluent samples were collected from the University Clinics of Kinshasa (CUK), a tertiary referral hospital established in 1957 by Lovanium University (now the University of Kinshasa) and located on the university campus behind the Faculty of Medicine. The CUK provides healthcare services, clinical training, and research activities. Initially designed for 1,000 beds, the hospital currently operates approximately 800 beds, of which 545 are functional across ten departments. Bed occupancy ranges from 50% to 70%, with average patient stays of 3 days in maternity wards and up to 17 days in other units.

Daily water consumption per bed (200–400 L) results in an estimated wastewater generation of 109,000–218,000 L/day (Lubieno, 2018). The hospital complex includes three former National Transport Office buildings and additional structures constructed by Lovanium University (Nguma, 2016, as cited in Lubieno, 2019).

Wastewater management at the CUK relies on a mixed drainage system combining combined and separate sewer networks. The original combined system consisted of three main collectors discharging into the Monastery Valley, the Funa River, and the Kemi River. At present, blackwater and technical effluent pipelines are largely non-functional. A partially operational separate system equipped with three septic tanks remains in place; however, these units are severely degraded. Currently, only stormwater is conveyed through the legacy sewer network.

UASB Reactor

General Principle

The UASB reactor, originally developed by [Lettinga et al. \(1980\)](#), is an anaerobic treatment system in which wastewater flows upward through a bed of anaerobic sludge, allowing efficient organic matter degradation and biogas production ([Liu et al., 2002](#)). The high settling capacity and structural stability of the sludge bed, combined with its ability to retain solids and certain heavy metals through adsorption and precipitation mechanisms, make UASB technology suitable for treating complex effluents such as hospital wastewater ([Singh et al., 2021](#); [Tchobanoglou et al., 2014](#)).

Experimental Setup

Two pilot-scale UASB reactors were constructed from transparent PVC (5 mm thickness) to allow visual observation of internal processes, including sludge behaviour and phase separation. The reactors were operated under two different conditions:

- **P14:** Reactor operated under optimised conditions
- **P4:** Reactor operated under non-optimised conditions (control)

Both reactors had a total volume of 2.5 L and a working volume of 2.0 L, with a height of 30 cm and a diameter of 6 cm. The upflow velocity was maintained at 1 m/h. Hydraulic retention time (HRT) was set at 8 h for the optimised reactor (P14) and 6 h for the non-optimised reactor (P4).

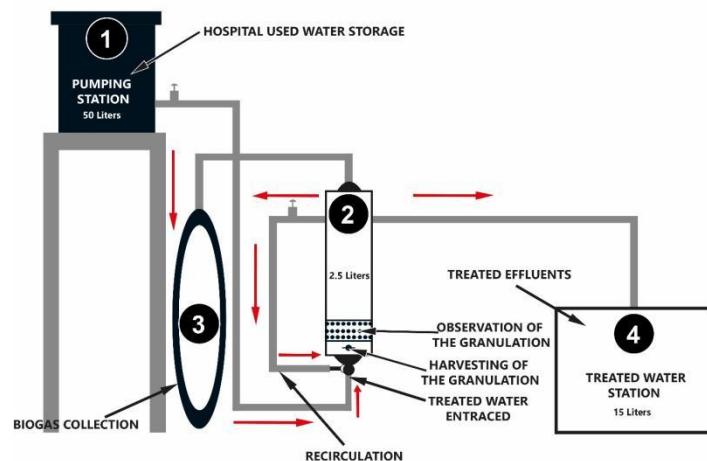
Reactor Configuration and Operating Conditions

Each UASB reactor consisted of four main components: (i) an influent distribution system equipped with a perforated diffuser to ensure uniform upward flow; (ii) an anaerobic reaction zone containing a granular or flocculent sludge bed; (iii) a settling zone to promote biomass-effluent separation; and (iv) a biogas collection chamber with an outlet tube allowing visual monitoring of gas production.

Operational parameters for reactor P14 were selected based on previous studies to promote stable anaerobic performance ([El-Gohary et al., 1995](#); [Latif et al., 2011](#); [Liu et al., 2003](#)). The pH was maintained at 7.0 ± 0.1 using NaOH or HCl, temperature was controlled at 35 ± 1 °C using a thermostatic water bath, and the influent C/N/P ratio was adjusted to 100:5:1. These conditions were not applied to reactor P4, which served as the non-optimised reference system.

The following diagrams illustrate the configurations of the non-optimized and optimized pilot-scale UASB reactors used in this study.

Figure 1:
Non-Optimized Pilot-Scale UASB Reactor
NO- OPTIMAL REACTOR



Description of the reactor configuration

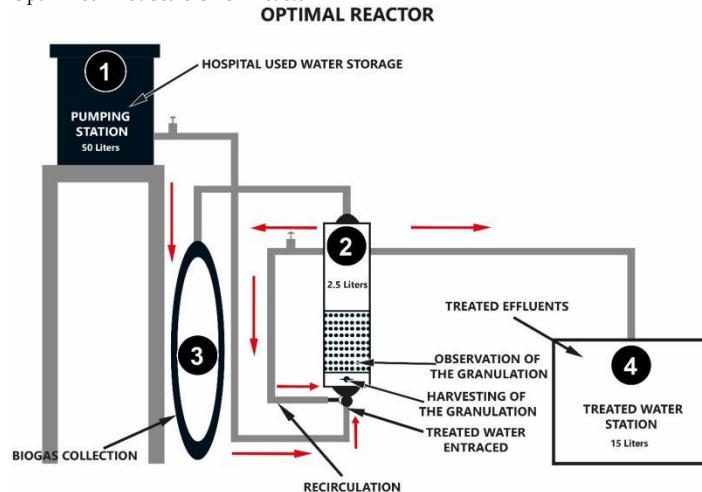
The non-optimized reactor system comprised four principal components, identified numerically in the schematic:

- (1) a raw wastewater storage tank;
- (2) a vertically oriented UASB reactor receiving influent at the base and containing an anaerobic sludge bed, with limited internal recirculation;
- (3) a gas collection chamber, although biogas production was negligible under the experimental conditions; and
- (4) a final effluent collection tank.

The UASB reactor included a perforated influent distributor at the bottom, a central reaction zone containing granular or flocculent anaerobic sludge, an upper settling zone designed to promote biomass-liquid separation, and a simplified gas trap that allowed visual observation of gas release.

Operational limitations were evident in the non-optimized configuration. These included biomass washout linked to hydraulic instability, a reduced hydraulic retention time (HRT) of approximately 6 h that limited effective substrate-biomass contact, and insufficient settling capacity leading to biomass loss and accumulation of volatile fatty acids. Biogas production was low and unstable, indicating suboptimal anaerobic process performance and reduced system stability.

Figure 2:
Optimized Pilot-Scale UASB Reactor



Description of the reactor configuration

The optimized treatment system also consisted of four main units:

- (1) a raw wastewater storage tank serving as the influent source;
- (2) a vertically oriented UASB reactor in which wastewater flowed upward through an anaerobic sludge bed, with enhanced internal recirculation to improve biomass-substrate contact;
- (3) a gas collection chamber for visual monitoring of biogas, although overall gas production remained limited during the experimental period; and
- (4) a treated effluent basin for clarified discharge.

The reactor was equipped with a perforated influent distributor at the base to ensure uniform flow distribution, a central anaerobic reaction zone containing well-retained granular sludge, an adequately dimensioned upper settling zone for effective solid-liquid separation, and a simplified gas trap for gas observation.

Under optimized operating conditions, the reactor was run at a controlled influent flow rate corresponding to an HRT of 8–12 h. The improved hydraulic regime and settling efficiency promoted effective biomass retention and stable reactor operation. Consistent gas release was observed, reflecting steady anaerobic activity and improved overall process stability compared to the non-optimized system.

Addition of Natural Additives

In the optimised reactor (P14), natural additives were introduced at start-up to enhance heavy metal retention, while their specific role in sludge granulation was beyond

the scope of this study. Clay (1.5%, w/w) was used for metal adsorption due to its high cation exchange capacity; crushed eggshells (1%, w/w) provided calcium carbonate buffering and promoted metal precipitation; ground maize (2%, w/w) acted as a slow-release carbon source facilitating biosorption; and hydrated lime (0.5%, w/w) was added to maintain mildly alkaline conditions (pH 8–9) conducive to metal hydroxide precipitation.

Additives were supplied daily from May to October to sustain removal efficiency, compensate for potential adsorbent saturation, and stabilise reactor performance. No additives were added to the control reactor (P4), allowing direct assessment of their contribution to heavy metal retention.

Sampling

Sampling was conducted weekly over a 12-week period at three locations: raw influent (P9), effluent from the non-optimised reactor (P4), and effluent from the optimised reactor (P14). At each sampling point, three independent replicates were collected per week, resulting in a total of 36 samples per point. Samples (500 mL) were pre-filtered through a 1 mm sieve, stored at 4 °C, and analysed within 24 h to minimise physicochemical changes.

Physicochemical Analyses

Physicochemical analyses focused on conventional water quality parameters and environmentally relevant heavy metals. All analytical procedures were conducted in accordance with *Standard Methods for the Examination of Water and Wastewater* (APHA, 2017).

Temperature was measured using a digital thermometer, pH was determined electrometrically with a calibrated Hanna HI98191 pH meter, and electrical conductivity was measured using a WTW Cond 3310 conductometer. Chemical oxygen demand (COD) was analysed by dichromate digestion followed by titration, according to APHA Method 5220-D.

Concentrations of Fe, Mn, Zn, Cu, Ni, and Al were determined by atomic absorption spectrometry (AAS) using a PerkinElmer AAnalyst 400. Samples were filtered through 0.45 µm membrane filters and acidified with nitric acid to pH < 2. Metal digestion was performed using a hot HNO₃/HCl (3:1, v/v) mixture. Calibration was carried out using certified reference standards (Merck TraceCERT). Duplicate analyses and analytical blanks were included for quality assurance.

Statistical Analysis

All data were processed using R statistical software (version 4.3). Data normality was assessed using the

Shapiro-Wilk test, and homogeneity of variances was verified using Levene's test. Differences among raw influent (P9), non-optimised reactor effluent (P4), and optimised reactor effluent (P14) were evaluated using one-way analysis of variance (ANOVA).

When significant differences were detected, Tukey's honestly significant difference (HSD) test was applied for post hoc comparisons. Pearson correlation coefficients were calculated to examine relationships between COD, pH, conductivity, and heavy metal concentrations. Multiple linear regression analysis was used to assess the influence of selected operational parameters on treatment performance. Statistical significance was set at $p < 0.05$.

RESULTS

Physicochemical Characterisation of Effluents

Table 1 presents the mean values \pm standard deviation (SD) and 95% confidence intervals (CI) of the physicochemical parameters measured at the three sampling points: raw hospital effluent (P9), effluent from the non-optimised UASB reactor (P4), and effluent from the optimised UASB reactor supplemented with natural additives (P14). World Health Organization (WHO, 2021) guideline values for discharge into aquatic environments are included for comparison.

Table 1:
Physicochemical characterisation of hospital wastewater at different treatment stages

Parameter	P9 (Raw)	P4 (Non-optimised)	P14 (Optimised)	WHO guideline (2021)
pH	7.25 \pm 0.15 (7.10–7.40)	7.10 \pm 0.12 (6.98–7.22)	7.05 \pm 0.10 (6.95–7.15)	6.5–8.5
Conductivity ($\mu\text{S}/\text{cm}$)	1850 \pm 200 (1660–2040)	1400 \pm 180 (1310–1490)	1250 \pm 150 (1160–1340)	< 2000
COD (mg/L)	620 \pm 35 (598–642)	280 \pm 25 (263–297)	150 \pm 20 (140–160)	< 50
Fe (mg/L)	1.20 \pm 0.10 (1.14–1.26)	0.90 \pm 0.08 (0.84–0.96)	0.45 \pm 0.05 (0.42–0.48)	0.3
Mn (mg/L)	0.35 \pm 0.04 (0.32–0.38)	0.25 \pm 0.03 (0.23–0.27)	0.10 \pm 0.02 (0.09–0.11)	0.1
Ni (mg/L)	0.18 \pm 0.02 (0.16–0.20)	0.12 \pm 0.01 (0.11–0.13)	0.05 \pm 0.01 (0.04–0.06)	0.02
Cu (mg/L)	0.50 \pm 0.05 (0.46–0.54)	0.35 \pm 0.04 (0.32–0.38)	0.15 \pm 0.02 (0.14–0.16)	2.0
Zn (mg/L)	0.85 \pm 0.07 (0.80–0.90)	0.60 \pm 0.05 (0.56–0.64)	0.30 \pm 0.03 (0.28–0.32)	3.0

Note: Values are mean \pm SD, with 95% confidence intervals in parentheses.

The optimised reactor (P14) achieved the highest reductions in COD (approximately 76%) and heavy metal concentrations, particularly for Fe, Cu, and Zn. Nickel and manganese concentrations were reduced but remained slightly above WHO guideline limits, indicating the need for additional post-treatment. pH values remained stable across all reactors (7.05–7.25), supporting favourable conditions for microbial activity and sludge stability. Conductivity decreased progressively along the treatment train, reflecting a reduction in dissolved ionic content.

Temporal Trends of COD and Heavy Metals

Temporal variations in COD were modelled using a first-order saturating exponential decay function:

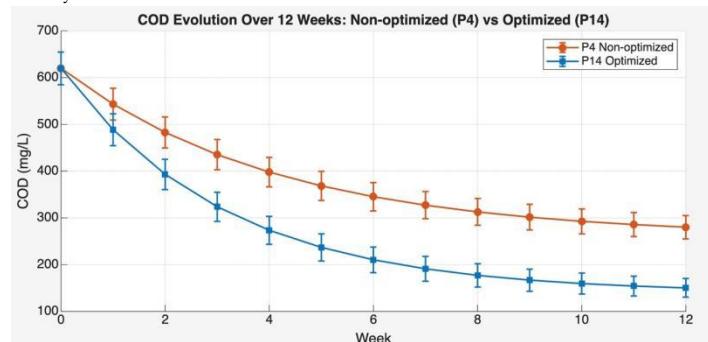
$$C(t) = K + (C_0 - K) e^{-kt}$$

where $C(t)$ is the COD concentration at time t (weeks), C_0 is the initial COD concentration (raw influent, 620 mg/L), K is the asymptotic plateau (residual COD), and k is the decay constant representing the rate of stabilisation.

Table 2:
Model parameters describing COD decay under non-optimised and optimised conditions

Parameter	Non-optimised (P4)	Optimised (P14)
Model form	$C_{p4}(t) = K_{p4} + (C_0 - K_{p4})e^{-k_{p4}t}$	$C_{p14}(t) = K_{p14} + (C_0 - K_{p14})e^{-k_{p14}t}$
Initial COD, C_0 (mg/L)	620	620
Plateau, K (mg/L)	260	140
Decay constant, k (week $^{-1}$)	0.24	0.32
Explicit model	$C_{p4}(t) = 260 + 360e^{-0.24t}$	$C_{p14}(t) = 140 + 480e^{-0.32t}$

Figure 1:
Weekly variation of COD concentrations over 12 weeks



To further characterise metal-specific dynamics, the same model structure was applied to individual heavy metals.

Common saturating model

$$C(t) = K + (C_0 - K)e^{-kt}$$

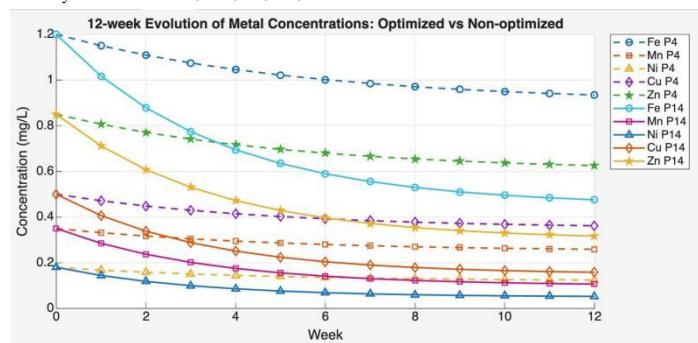
Table 3:

Metal-specific temporal decay models under non-optimised and optimised conditions

Metal	Non-optimised reactor (P4)	Optimised reactor (P14)
Fe	$C_{Fe,P4}(t) = 0.90 + 0.30e^{-0.18t}$	$C_{Fe,P14}(t) = 0.45 + 0.75e^{-0.28t}$
Mn	$C_{Mn,P4}(t) = 0.25 + 0.10e^{-0.20t}$	$C_{Mn,P14}(t) = 0.10 + 0.25e^{-0.30t}$
Ni	$C_{Ni,P4}(t) = 0.12 + 0.06e^{-0.22t}$	$C_{Ni,P14}(t) = 0.05 + 0.13e^{-0.32t}$
Cu	$C_{Cu,P4}(t) = 0.35 + 0.15e^{-0.21t}$	$C_{Cu,P14}(t) = 0.15 + 0.35e^{-0.31t}$
Zn	$C_{Zn,P4}(t) = 0.60 + 0.25e^{-0.19t}$	$C_{Zn,P14}(t) = 0.30 + 0.55e^{-0.29t}$

Figure 2:

Weekly variation of Fe, Mn, Ni, Cu, and Zn concentrations over 12 weeks



COD and metal concentrations decreased steadily in the optimised reactor, with no abrupt loss of performance. In contrast, the non-optimised reactor exhibited slower removal rates and occasional fluctuations, likely associated with biomass washout and insufficient hydraulic retention time. Continuous additive supplementation maintained treatment efficiency, suggesting minimal adsorbent saturation during the 12-week period.

Statistical Analysis

Table 4:

One-way ANOVA results and effect sizes for COD and heavy metals

Parameter	F	p-value	η^2 (effect size)
COD	112.4	<.001	0.68
Fe	95.3	<.001	0.64
Mn	88.1	<.001	0.62
Ni	75.5	<.001	0.58
Cu	80.2	<.001	0.59
Zn	90.4	<.001	0.63

Highly significant differences ($p < .001$) were observed among the three sampling points for all parameters, with large effect sizes ($\eta^2 > 0.5$). Tukey's HSD post hoc analysis confirmed that effluent quality from the optimised reactor (P14) differed significantly from both the raw influent (P9) and the non-optimised reactor (P4).

Regression Analysis and Correlations

Table 5:

Multiple linear regression between COD and heavy metal concentrations

Metal	Predictor	Coefficient \pm SE	p-value	R ²
Fe	COD	0.85 ± 0.05	<.001	0.72
Mn	COD	0.78 ± 0.06	<.001	0.61
Ni	COD	0.65 ± 0.07	.002	0.50
Cu	COD	0.82 ± 0.05	<.001	0.68
Zn	COD	0.80 ± 0.05	<.001	0.66

COD reduction showed strong positive correlations with metal removal, supporting the role of combined adsorption, precipitation, and biofilm-mediated mechanisms within the UASB reactor.

Mass Balance of Heavy Metals

Table 6:

Simple mass balance for selected metals in the optimised reactor (P14) over 12 weeks

Metal	Influent load (mg)	Effluent load (mg)	Removal (%)
Fe	7,440	2,790	62.5
Ni	1,116	310	72.2
Cu	3,700	1,110	70.0

The mass balance confirms substantial retention of heavy metals within the reactor, with removal efficiencies exceeding 60% for all analysed metals. Residual concentrations indicate the need for post-treatment to fully comply with WHO discharge standards.

Summary of Results

The optimised UASB reactor consistently outperformed the non-optimised system in reducing COD and heavy metal concentrations. Nevertheless, residual levels of COD, iron, nickel, and manganese remained above WHO discharge limits, underscoring the necessity for complementary post-treatment processes. The strong statistical associations observed between COD and metal removal support the effectiveness of anaerobic treatment as a primary treatment step, while highlighting the

importance of integrated treatment strategies to achieve full environmental compliance.

DISCUSSION

Enhanced Treatment Performance in the Optimised UASB Reactor

This study demonstrates that optimisation of UASB reactor operating conditions, particularly through the incorporation of natural materials such as clay, crushed eggshells, and maize, significantly enhances the treatment of hospital effluents from the University Clinics of Kinshasa (CUK). Chemical oxygen demand (COD) decreased from 620 mg/L in the raw influent to 150 mg/L in the optimised reactor, in agreement with previous reports on the effectiveness of UASB systems for anaerobic degradation of complex organic matter (Giménez et al., 2011; Lettinga et al., 1980).

While improved organic matter removal was observed, the primary focus of this study was heavy metal removal. The effects of optimisation on microbial granulation, biomass development, and long-term sludge stability were not investigated and will be addressed in subsequent work.

Role of Natural Additives in Heavy Metal Removal

The addition of clay, crushed eggshells, ground maize, and hydrated lime in the optimised reactor substantially enhanced heavy metal removal through a combination of adsorption, precipitation, and complexation mechanisms (Kalyuzhnyi et al., 2006; Verlicchi et al., 2010). The high surface area, cation exchange capacity, and carbonate content of these materials facilitated the retention of Fe, Mn, Ni, and Cu within the reactor system.

Heavy metal concentrations in the optimised reactor (P14) were markedly lower than those observed in the non-optimised reactor (P4) and raw influent (P9), indicating a synergistic interaction between anaerobic biomass and the natural additives. In addition to improving metal retention, these materials likely reduced metal toxicity, thereby supporting the stability and activity of anaerobic microbial communities essential for effective wastewater treatment (Li et al., 2019; Wang et al., 2020).

The experimental dataset consisted only of initial concentrations (raw influent, P9) and final values measured at week 12 for both reactors (P4 and P14). This limited temporal resolution prevented direct observation of intermediate concentration dynamics. To address this limitation, MATLAB was used to generate continuous concentration profiles by fitting a theoretical saturating model to the available boundary values. The model then predicted intermediate concentrations in a physically consistent manner.

This modelling approach highlights an inherent limitation of the experimental design. The absence of intermediate sampling points restricts the ability to capture the true temporal behaviour of COD and heavy metal removal. Consequently, future studies should incorporate systematic sampling at multiple time points to allow direct validation of kinetic models and to provide a more detailed understanding of treatment dynamics.

Biochemical Correlations and Implications for Treatment

Statistical analyses revealed strong positive correlations between COD reduction and heavy metal removal, particularly for iron ($r = 0.85$), suggesting that organic matter degradation may facilitate metal retention through combined biological and physicochemical processes. The slight acidification observed in the optimised reactor may have promoted controlled metal solubilisation followed by precipitation or adsorption onto sludge and additives (Li et al., 2019).

Despite the overall improvement in treatment performance, residual concentrations of certain metals, notably nickel and manganese, remained above WHO discharge limits. This finding indicates that, although natural additives significantly enhance removal efficiency, additional measures such as extended hydraulic retention times, sequential adsorption stages, or post-treatment processes (e.g., activated carbon filtration or advanced oxidation) are required to achieve full regulatory compliance.

Limitations and Future Perspectives

This study represents an initial evaluation of heavy metal removal using UASB reactors supplemented with natural additives. Several limitations should be acknowledged. First, the experimental duration of 12 weeks may not adequately capture long-term variations in influent composition or treatment stability. Second, the use of small pilot-scale reactors (2.5 L) limits direct extrapolation of results to full-scale applications. Third, detailed microbial analyses were not conducted, and microbial granulation and community structure were not assessed.

Future research should involve larger-scale and longer-term trials to validate the observed performance under more representative operating conditions. Advanced microbiological techniques, such as metagenomic sequencing, should be employed to elucidate interactions between natural additives, heavy metals, and anaerobic microbial communities (Wang et al., 2020). In addition, integration of the optimised UASB reactor with complementary treatment processes, including adsorption or advanced oxidation, may be necessary to ensure

complete contaminant removal and compliance with environmental standards.

Concluding Remarks on Feasibility

Despite the limitations identified, the findings indicate that the combination of optimised UASB operation and natural additives constitutes a promising primary treatment strategy for hospital wastewater. The approach substantially reduces organic and metal loads while maintaining conditions favourable for anaerobic microbial activity. However, additional treatment steps are required to fully meet international discharge standards.

CONCLUSION

Optimisation of UASB reactor operation through the use of natural additives, including clay, crushed eggshells, maize, and hydrated lime, effectively reduced pollution in hospital effluents from the University Clinics of Kinshasa. COD decreased from 620 ± 35 mg/L in the raw influent to 150 ± 20 mg/L in the optimised reactor, corresponding to an approximate removal efficiency of 76%. Concentrations of heavy metals, including Fe, Mn, Ni, Zn, and Cu, were significantly reduced, with iron levels halved and zinc and copper concentrations meeting WHO guideline limits.

Statistical analyses, including ANOVA and Tukey's HSD tests, confirmed the significance of these improvements. Strong correlations between COD reduction and metal removal indicate that adsorption, precipitation, and bio-assisted mechanisms collectively contributed to metal retention within the reactor system.

The study focused primarily on heavy metal removal; microbial granulation and community dynamics were not investigated and will be addressed in future research. Residual concentrations of nickel and manganese remained above WHO discharge limits, highlighting the need for additional post-treatment processes. Future work should prioritise long-term monitoring, scale-up to pilot or full-scale systems, integration with complementary treatment technologies, and molecular characterisation of microbial communities to better understand interactions between additives and anaerobic biomass.

Overall, these findings provide a strong foundation for sustainable hospital wastewater management in resource-limited settings, demonstrating that optimised UASB systems supplemented with natural additives can substantially reduce organic load and heavy metal contamination while supporting stable anaerobic treatment processes.

Ethical Approval: Nil required.

Conflicts of Interest: None declared.

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