International Journal of Research and Technology (IJRT)
International Open-Access, Peer-Reviewed, Refereed, Online Journal
ISSN (Print): 2321-7510 | ISSN (Online): 2321-7529
| An ISO 9001:2015 Certified Journal |

Intelligent Bioremediation In The Industry 5.0 Era

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ABSTRACT

Background: Conventional bioremediation approaches suffer from unpredictable efficacy, slow kinetics, and lack of real-time adaptive control in complex industrial environments. The convergence of Industry 5.0 technologies with advanced biotechnology presents an unprecedented opportunity to create autonomous, self-optimizing remediation systems.

Objective: This study aimed to develop and validate an integrated intelligent bioremediation platform combining (i) AI-guided synthetic microbial consortia design, (ii) IoT-enabled biosensor networks for real-time monitoring, and (iii) adaptive process control algorithms for autonomous optimization of mixed pollutant degradation.

Methods: A synthetic microbial consortium comprising three engineered strains (Pseudomonas putida KT2440-AB, Rhodococcus erythropolis PR4-PAH, and Cupriavidus metallidurans CH34-HM) was constructed using metabolic engineering and validated for simultaneous degradation of hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and heavy metals. A Random Forest-based AI model (RF-BioOpt) was trained on 357 experimental data points to predict optimal process parameters. An IoT biosensor array monitoring pH, dissolved oxygen, ORP, and pollutant concentrations was integrated with a programmable logic controller (PLC) for adaptive feedback control. Performance was evaluated in 15 L sequencing batch bioreactors treating simulated industrial wastewater over 45 operational cycles.

Results: The AI-optimized consortium achieved 94.3% total petroleum hydrocarbon (TPH) degradation, 89.7% PAH removal, and 82.5% Pb^{2+} biosorption within 72 hours under AI-determined optimal conditions (C/N ratio 16.4:1, DO 3.8 mg/L, pH 7.2, temperature 32.5°C). Real-time IoT monitoring enabled dynamic adjustment of aeration and nutrient dosing, reducing operational costs by 31.2% compared to fixed-parameter operation. The RF-BioOpt model demonstrated high predictive accuracy (R^2 = 0.978, RMSE = 0.042). Metagenomic analysis revealed stable consortium composition with <5% population drift over 45 cycles.

Conclusion: The integrated intelligent bioremediation platform successfully demonstrated autonomous, efficient, and stable degradation of mixed industrial pollutants, validating the Industry 5.0 paradigm of human-centric, sustainable, and resilient environmental biotechnology. This study provides a scalable framework for next-generation industrial wastewater treatment and contaminated site restoration.

Keywords: Intelligent bioremediation; Industry 5.0; synthetic microbial consortia; artificial intelligence; IoT biosensors; adaptive process control; mixed pollutants

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1. INTRODUCTION

The unprecedented escalation of industrial activities, urbanization, and agricultural intensification has precipitated a global environmental crisis characterized by the continuous release of complex, multi-component pollutant mixtures into soil and water ecosystems [1]. Industrial effluents typically contain heterogeneous combinations of petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and heavy metals, which exhibit synergistic toxicity and recalcitrance to conventional treatment approaches [2]. Traditional physico-chemical remediation methods, including advanced oxidation processes, chemical precipitation, and activated carbon adsorption, while effective in controlled settings, are frequently compromised by prohibitive operational costs, high energy consumption, generation of secondary hazardous waste, and ecological disruption when applied at industrial scales [3].

Bioremediation, which harnesses the innate metabolic capabilities of microorganisms and their enzymatic machinery, has been extensively investigated as a sustainable, cost-effective, and environmentally compatible alternative [4]. However, conventional bioremediation strategies—including bioaugmentation with single-strain inoculants and biostimulation through nutrient amendment—exhibit fundamental limitations that constrain their industrial applicability. These include unpredictable degradation kinetics in complex environmental matrices, competitive exclusion of introduced strains by indigenous microbiota, substrate inhibition at high pollutant concentrations, and a critical absence of real-time monitoring and adaptive control mechanisms [5].

The advent of Industry 5.0, a paradigmatic evolution beyond the automation-focused Industry 4.0 framework, introduces a novel operational philosophy anchored in three foundational pillars: human-centricity, environmental sustainability, and systemic resilience [6]. This conceptual framework provides the requisite scaffolding for developing next-generation bioremediation technologies that transcend the limitations of conventional approaches. The convergence of artificial intelligence (AI), Internet of Things (IoT) sensor networks, and synthetic biology within the Industry 5.0 paradigm enables the creation of cyber-biological systems wherein biological pollutant degradation is continuously monitored, computationally modeled, and autonomously optimized [7].

Recent advances in synthetic biology and metabolic engineering have substantially expanded the repertoire of microbial biocatalysts available for environmental applications [8]. Engineered strains with enhanced substrate specificity, broadened metabolic capabilities, and improved stress tolerance can now be rationally designed and constructed. Furthermore, the development of synthetic microbial consortia—engineered communities comprising complementary metabolic specialists—has emerged as a promising strategy for addressing multi-component pollutant mixtures that cannot be effectively degraded by single-strain inoculants [9]. Parallel advances in AI and machine learning (ML) have demonstrated remarkable efficacy in predicting optimal bioremediation conditions, modeling complex microbial interactions, and enabling inverse design of treatment protocols [10].

Despite these individual technological advancements, the systematic integration of AI-guided synthetic biology, IoT-enabled real-time monitoring, and adaptive process control into a unified, autonomous bioremediation platform has not been experimentally demonstrated. This represents a critical translational gap between laboratory-scale proof-of-concept studies and field-deployable intelligent remediation systems.

The present study was designed to address this knowledge gap through the following specific objectives: (i) construction and validation of an engineered synthetic microbial consortium capable of simultaneous hydrocarbon, PAH, and heavy metal degradation; (ii) development of an AI-based predictive model (RF-BioOpt) trained on experimental data to identify optimal process parameters; (iii) integration of IoT biosensor networks with programmable logic controllers for real-time adaptive feedback control; and (iv) performance evaluation of the integrated intelligent bioremediation platform under simulated industrial wastewater conditions. We hypothesized that the integrated platform would achieve significantly higher pollutant removal efficiencies, enhanced operational stability, and reduced resource consumption compared to conventional fixed-parameter bioremediation approaches.

2. MATERIALS AND METHODS

2.1. Chemicals, Reagents, and Culture Media

All chemicals and reagents used in this study were of analytical grade and procured from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise specified. Petroleum hydrocarbon standards (C10–C40 mixture) and PAH standards (naphthalene, phenanthrene, pyrene, benzo[a]pyrene) were obtained from Supelco (Bellefonte, PA, USA). Heavy metal stock solutions ($Pb(NO_3)_2$, $CdCl_2$, $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$) were prepared in deionized water at 10,000 mg/L and sterilized by filtration (0.22 µm membrane, Millipore, USA).

Luria-Bertani (LB) broth and agar were used for routine bacterial cultivation. Minimal salt medium (MSM) was prepared according to the following composition (per liter): K_2HPO_4 4.35 g, KH_2PO_4 1.70 g, NH_4Cl 2.10 g, $MgSO_4 \cdot 7H_2O$ 0.20 g, $MnSO_4 \cdot H_2O$ 0.05 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, $CaCl_2 \cdot 2H_2O$ 0.03 g, and trace element solution 1.0 mL. The pH was adjusted to 7.2 ± 0.1 using 1 M NaOH or HCl. Simulated industrial wastewater (SIWW) was prepared by supplementing MSM with diesel fuel (commercial grade, 5,000 mg/L), PAH mixture (50 mg/L each of naphthalene, phenanthrene, pyrene, and benzo[a]pyrene), and heavy metal mixture (Pb^{2+} 100 mg/L, Cd^{2+} 20 mg/L, Cu^{2+} 50 mg/L, Zn^{2+} 80 mg/L) [11].

2.2. Bacterial Strains and Genetic Engineering

Three bacterial strains were selected as chassis organisms for constructing the synthetic microbial consortium based on their well-characterized metabolic capabilities and genetic tractability: Pseudomonas putida KT2440 (ATCC 47054) for hydrocarbon degradation [12]; Rhodococcus erythropolis PR4 (JCM 16278) for PAH catabolism [13]; and Cupriavidus metallidurans CH34 (ATCC 43123) for heavy metal biosorption and sequestration [14].

P. putida KT2440 was engineered for enhanced alkane degradation through chromosomal integration of the alkBFGHJKL operon from P. putida GPo1 under the control of the constitutive Ptac promoter. The alkS regulatory gene was deleted to achieve constitutive expression. Genomic integration was performed using the suicide plasmid pK18mobsacB and verified by colony PCR and Sanger sequencing [15].

R. erythropolis PR4 was engineered for broad-spectrum PAH degradation through heterologous expression of the nidAB and phdEFGHIJK gene clusters from Mycobacterium vanbaalenii PYR-1, encoding ring-hydroxylating dioxygenases and extradiol dioxygenases, respectively. The gene clusters were assembled into the pTip-QC1 expression vector and introduced via electroporation [16].

C. metallidurans CH34 was engineered for enhanced Pb^{2+} biosorption by overexpression of the endogenous pbr operon (pbrR-pbrABCD) and heterologous expression of the mtA metallothionein gene from Synechococcus sp. PCC 7942 under the control of the Pb^{2+}-inducible PzntA promoter. The engineered cassette was integrated into the chromosome using the mini-Tn7 transposon system [17].

2.3. Construction and Optimization of Synthetic Microbial Consortium

The three engineered strains were cultivated separately in LB broth at 30°C with shaking (180 rpm) until late exponential phase (OD_{600} = 0.8–1.0). Cells were harvested by centrifugation (6,000 × g, 10 min, 4°C), washed twice with sterile phosphate-buffered saline (PBS, pH 7.2), and resuspended in MSM to a final concentration of 1×10^9 CFU/mL for each strain.

Initial consortium composition was determined using a simplex-centroid mixture design with three components (strains) and evaluated at seven inoculation ratios. The design matrix included pure strain controls (1:0:0, 0:1:0, 0:0:1), binary combinations (1:1:0, 1:0:1, 0:1:1), and the ternary combination (1:1:1). Performance was assessed by measuring TPH degradation, PAH removal, and Pb^{2+} biosorption after 72 h incubation in SIWW at 30°C. The optimal consortium composition was identified through response surface methodology (RSM) using Design-Expert software (Version 13, Stat-Ease, USA).

2.4. AI Model Development and Training

The Random Forest-based BioOpt model (RF-BioOpt) was developed for predicting pollutant degradation efficiency and identifying optimal process parameters. The dataset comprised 357 experimental data points collected from preliminary studies and literature mining [18], encompassing the following input features: initial TPH concentration (500–10,000 mg/L), initial PAH concentration (10–100 mg/L), initial heavy metal concentration (50–500 mg/L), C/N ratio (5:1 to 30:1), dissolved oxygen (1.0–6.0 mg/L), pH (5.5–8.5), temperature (20–40°C), and consortium inoculation ratio.

The dataset was randomly partitioned into training (70%, n = 250), validation (15%, n = 53), and test (15%, n = 54) sets. The Random Forest model was implemented using the scikit-learn library (Version 1.2.0) in Python 3.9. Hyperparameter optimization was performed using grid search with 5-fold cross-validation, evaluating combinations of the following parameters: number of trees (n_estimators) = [100, 200, 300, 500], maximum tree depth (max_depth) = [5, 10, 15, 20, None], minimum samples per split (min_samples_split) = [2, 5, 10], and minimum samples per leaf (min_samples_leaf) = [1, 2, 4]. Model performance was evaluated using coefficient of determination (R^2), root mean square error (RMSE), and mean absolute error (MAE). Feature importance was assessed using Gini impurity-based importance scores and SHAP (SHapley Additive exPlanations) values.

2.5. IoT Biosensor Network and Adaptive Control System

A multi-parameter IoT monitoring system was designed and constructed for real-time surveillance of bioreactor conditions (Fig. 1). The sensor array comprised: (i) optical dissolved oxygen sensor (InPro 6860i, Mettler Toledo, Switzerland); (ii) pH electrode (InPro 3250i, Mettler Toledo); (iii) oxidation-reduction potential (ORP) sensor (Pt4805-DXK, Mettler Toledo); (iv) turbidity sensor (InPro 8200, Mettler Toledo); and (v) online total organic carbon (TOC) analyzer (Sievers M9, SUEZ, USA). All sensors were connected to a multi-channel transmitter (M800, Mettler Toledo) with Modbus TCP/IP communication protocol.

For real-time heavy metal monitoring, a custom electrochemical biosensor was developed based on screen-printed carbon electrodes modified with Pb^{2+}-specific DNAzyme and gold nanoparticles [19]. The biosensor exhibited a linear detection range of 5–500 µg/L Pb^{2+} (R^2 = 0.994) and a detection limit of 2.3 µg/L.

Sensor data were transmitted to a cloud-based IoT platform (AWS IoT Core) via a Raspberry Pi 4 gateway at 30-second intervals. A programmable logic controller (PLC; Siemens S7-1200) received processed data and executed adaptive control algorithms for aeration rate adjustment (0.5–5.0 L/min), nutrient dosing (NH_4Cl and K_2HPO_4 solutions), and pH correction (1 M NaOH/HCl). The control logic was implemented using a proportional-integral-derivative (PID) algorithm with real-time setpoint optimization based on RF-BioOpt model predictions.

2.6. Bioreactor Operation and Experimental Design

Bioremediation experiments were conducted in custom-designed 15 L sequencing batch bioreactors (SBRs) equipped with the IoT sensor network and adaptive control system. The SBRs were operated in 72-hour cycles comprising the following phases: fill (30 min), react (68 h), settle (2 h), draw (30 min), and idle (1 h). Four experimental conditions were evaluated in triplicate:

- **Condition A (Conventional Fixed-Parameter)**: Fixed C/N ratio 10:1, DO 2.5 mg/L, pH 7.0, temperature 30°C; manual sampling every 12 h.
- **Condition B (AI-Optimized Fixed-Parameter)**: RF-BioOpt-determined optimal parameters (C/N ratio 16.4:1, DO 3.8 mg/L, pH 7.2, temperature 32.5°C); manual sampling.
- **Condition C (AI + IoT Adaptive Control)**: Real-time adaptive control based on IoT sensor feedback and RF-BioOpt model predictions; automated aeration and nutrient adjustments.
- **Condition D (Abiotic Control)**: Sterile SIWW without microbial inoculation; identical conditions to Condition A.

Each bioreactor was inoculated with the optimized synthetic consortium at 1×10^8 CFU/mL total cell density. Samples were collected at 0, 12, 24, 36, 48, 60, and 72 h for analysis of pollutant concentrations, microbial growth, and enzyme activities.

2.7. Analytical Methods

Total Petroleum Hydrocarbons (TPH): TPH concentrations were determined by gas chromatography with flame ionization detection (GC-FID; Agilent 7890B) following liquid-liquid extraction with n-hexane. The GC was equipped with a DB-5MS column (30 m × 0.25

mm × 0.25 μm) and operated with the following temperature program: 50°C (2 min), ramp to 320°C at 8°C/min, hold 10 min [20].

PAH Analysis: PAH concentrations were quantified by high-performance liquid chromatography (HPLC; Agilent 1260 Infinity II) with UV-visible diode array detection (DAD) and fluorescence detection (FLD). Separation was achieved on a ZORBAX Eclipse PAH column (4.6 × 150 mm, 3.5 μm) with acetonitrile-water gradient elution [21].

Heavy Metal Analysis: Pb^{2+}, Cd^{2+}, Cu^{2+}, and Zn^{2+} concentrations were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; PerkinElmer Optima 8300). Samples were acidified with 2% HNO_3 and filtered (0.45 μm) prior to analysis.

Microbial Growth: Cell density was monitored by optical density at 600 nm (OD_{600}) using a UV-Vis spectrophotometer (Shimadzu UV-1800) and by colony-forming unit (CFU) enumeration on selective agar plates.

Enzyme Activities: Alkane hydroxylase activity was measured by NADH oxidation assay [22]; PAH dioxygenase activity was determined by indole oxidation assay [23]; and Pb^{2+} biosorption capacity was quantified by ICP-OES analysis of cell-bound metal following acid digestion.

2.8. Metagenomic Analysis

Microbial community dynamics were assessed by 16S rRNA gene amplicon sequencing. Genomic DNA was extracted from bioreactor samples (0, 24, 48, and 72 h of each cycle) using the DNeasy PowerSoil Pro Kit (Qiagen, Germany). The V3–V4 hypervariable region was amplified using primers 341F (5‘-CCTACGGGNGGCWGCAG-3’) and 805R (5‘-GACTACHVGGGTATCTAATCC-3’) and sequenced on an Illumina MiSeq platform (2 × 300 bp paired-end). Sequence data were processed using QIIME2 (Version 2023.5) with DADA2 for amplicon sequence variant (ASV) inference and SILVA 138.1 for taxonomic classification [24].

2.9. Statistical Analysis

All experiments were performed in triplicate and results are expressed as mean ± standard deviation. Statistical comparisons between treatment conditions were performed using one-way analysis of variance (ANOVA) followed by Tukey‘s honestly significant difference (HSD) post-hoc test. Differences were considered statistically significant at $p < 0.05$. Data analysis and visualization were conducted using Python 3.9 with pandas, NumPy, SciPy, and Matplotlib libraries.

3. RESULTS

3.1. Construction and Optimization of Synthetic Microbial Consortium

The three engineered strains were successfully constructed and validated for their respective degradation capabilities. P. putida KT2440-AB exhibited constitutive alkane hydroxylase activity of 2.47 ± 0.18 μmol NADH oxidized min^{-1} mg $protein^{-1}$, representing a 4.3-fold increase compared to the wild-type strain ($p < 0.001$). R. erythropolis PR4-PAH demonstrated PAH dioxygenase activity of 156.8 ± 12.3 nmol indigo formed min^{-1} mg $protein^{-1}$ against phenanthrene as substrate. C. metallidurans CH34-HM displayed Pb^{2+} biosorption capacity of

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287.4 ± 21.6 mg Pb^{2+} per gram dry cell weight, a 2.8-fold enhancement over the parental strain ($p < 0.001$).

Simplex-centroid mixture design experiments revealed significant synergistic interactions among the three strains for mixed pollutant degradation (Table 1). The optimal consortium composition was determined to be 45% P. putida KT2440-AB, 30% R. erythropolis PR4-PAH, and 25% C. metallidurans CH34-HM, which achieved 91.6% TPH degradation, 86.4% total PAH removal, and 78.9% Pb^{2+} biosorption after 72 h. The ternary consortium significantly outperformed all pure strain and binary combination treatments ($p < 0.001$), confirming the presence of positive metabolic interactions.

Table 1: Performance of Synthetic Microbial Consortium Compositions for Mixed Pollutant Degradation (72 h)

Consortium Composition (% v/v)	TPH Degradation (%)	Total PAH Removal (%)	Pb^{2+} Biosorption (%)
Pp 100%	72.3 ± 3.8	28.7 ± 2.4	12.4 ± 1.6
Re 100%	18.6 ± 2.1	73.5 ± 4.2	8.7 ± 1.2
Cm 100%	9.4 ± 1.3	11.2 ± 1.8	54.6 ± 3.7
Pp:Re 50:50	79.8 ± 4.2	81.2 ± 3.9	23.5 ± 2.1
Pp:Cm 50:50	74.5 ± 3.6	31.4 ± 2.7	59.8 ± 3.4
Re:Cm 50:50	21.3 ± 2.4	76.8 ± 3.5	62.3 ± 3.1
Pp:Re:Cm 33:33:33	88.7 ± 3.1	84.2 ± 3.3	74.5 ± 2.8
Pp:Re:Cm 45:30:25 (Optimal)	**91.6 ± 2.8**	**86.4 ± 3.1**	**78.9 ± 2.5**

Data represent mean ± SD of triplicate experiments. Pp: Pseudomonas putida KT2440-AB; Re: Rhodococcus erythropolis PR4-PAH; Cm: Cupriavidus metallidurans CH34-HM.

3.2. AI Model Performance and Process Optimization

The RF-BioOpt model demonstrated excellent predictive performance on the independent test dataset (n = 54). The optimized Random Forest configuration (n_estimators = 300, max_depth = 15, min_samples_split = 5, min_samples_leaf = 2) achieved $R^2 = 0.978$, RMSE = 0.042, and MAE = 0.031 for predicting overall pollutant removal efficiency (Fig. 2). Feature importance analysis using SHAP values identified initial TPH concentration, C/N ratio, and dissolved oxygen as the three most influential parameters, contributing 24.7%, 18.3%, and 15.6% to model predictions, respectively.

Model optimization identified the following optimal process conditions for maximum pollutant removal: C/N ratio = 16.4:1, dissolved oxygen = 3.8 mg/L, pH = 7.2, temperature = 32.5°C, and consortium inoculation ratio = 45:30:25 (Pp:Re:Cm). Under these AI-optimized conditions, predicted removal efficiencies were 94.8% for TPH, 90.2% for total PAHs, and 83.1% for Pb^{2+}.

Table 2: RF-BioOpt Model Performance Metrics and Optimal Process Parameters

Parameter	Value
Model Performance	
Training R^2	0.989
Validation R^2	0.982
Test R^2	0.978
Test RMSE	0.042
Test MAE	0.031
Optimal Process Parameters	
C/N ratio	16.4:1
Dissolved oxygen (mg/L)	3.8
pH	7.2
Temperature (°C)	32.5
Inoculation ratio (Pp:Re:Cm)	45:30:25
Predicted Removal Efficiency	
TPH (%)	94.8
Total PAHs (%)	90.2
Pb^{2+} (%)	83.1

3.3. Bioreactor Performance Under Different Operational Conditions

The integrated intelligent bioremediation platform (Condition C) demonstrated significantly superior pollutant removal performance compared to both conventional (Condition A) and AI-optimized fixed-parameter (Condition B) operations (Table 3).

Under adaptive control (Condition C), TPH degradation reached 94.3 ± 2.1% within 72 h, compared to 86.7 ± 3.4% in Condition B and 71.5 ± 4.2% in Condition A ($p < 0.001$). Similarly, total PAH removal was 89.7 ± 2.8% (Condition C), 82.4 ± 3.6% (Condition B), and 64.3 ± 4.1% (Condition A). Pb^{2+} biosorption achieved 82.5 ± 2.9% under adaptive control, representing significant improvements over both fixed-parameter conditions ($p < 0.001$). Abiotic controls (Condition D) showed negligible pollutant removal (<3%), confirming biological mechanisms as the primary degradation pathway.

Table 3: Comparative Pollutant Removal Efficiency After 72-Hour Bioreactor Operation

Condition	TPH Degradation (%)	Total PAH Removal (%)	Pb^{2+} Biosorption (%)	Cd^{2+} Removal (%)	Cu^{2+} Removal (%)	Zn^{2+} Removal (%)
A (Conventional Fixed)	71.5 ± 4.2[a]	64.3 ± 4.1[a]	54.8 ± 3.7[a]	48.2 ± 3.9[a]	52.6 ± 4.3[a]	45.7 ± 3.5[a]
B (AI-Optimized Fixed)	86.7 ± 3.4[b]	82.4 ± 3.6[b]	74.2 ± 3.1[b]	67.5 ± 3.4[b]	71.3 ± 3.8[b]	63.9 ± 3.2[b]

C (AI + IoT Adaptive)	94.3 ± 2.1^c	89.7 ± 2.8^c	82.5 ± 2.9^c	76.8 ± 2.6^c	79.4 ± 3.1^c	72.1 ± 2.8^c
D (Abiotic Control)	2.1 ± 0.5^d	1.8 ± 0.4^d	1.2 ± 0.3^d	0.9 ± 0.2^d	1.5 ± 0.4^d	1.1 ± 0.3^d

Data represent mean ± SD of triplicate 15 L bioreactor runs (45 operational cycles per condition). Different superscript letters within columns indicate statistically significant differences ($p < 0.05$, one-way ANOVA with Tukey HSD post-hoc test).

3.4. Real-Time Monitoring and Adaptive Control Dynamics

The IoT biosensor network successfully enabled continuous, real-time monitoring of critical bioreactor parameters throughout the 72-hour treatment cycles. Fig. 3 illustrates representative sensor trajectories during a single operational cycle under adaptive control (Condition C).

Dissolved oxygen concentrations were maintained at the AI-optimized setpoint of 3.8 ± 0.3 mg/L through dynamic adjustment of aeration rates (0.8–4.2 L/min). The PLC-controlled PID algorithm responded to transient DO fluctuations arising from variable microbial respiratory activity during different growth phases. pH values were maintained at 7.2 ± 0.15 through automated addition of 1 M NaOH, compensating for acidification associated with hydrocarbon oxidation intermediates.

The electrochemical Pb^{2+} biosensor provided real-time quantification of heavy metal removal kinetics, revealing first-order biosorption dynamics with a rate constant of $0.078 \pm 0.006\ h^{-1}$. Online TOC measurements correlated strongly with GC-FID TPH quantification ($R^2 = 0.943$), validating the use of TOC as a rapid proxy for hydrocarbon removal monitoring.

Table 4: IoT Biosensor Network Performance Characteristics

Sensor Parameter	Measurement Range	Accuracy	Response Time	Correlation with Reference Method (R^2)
Dissolved Oxygen	0–20 mg/L	±0.1 mg/L	<30 s	0.997 (optical reference)
pH	0–14	±0.05	<20 s	0.995 (electrode reference)
ORP	-1500 to +1500 mV	±5 mV	<30 s	N/A
Turbidity	0–4000 NTU	±2%	<10 s	0.972 (OD_{600})
Pb^{2+} Biosensor	5–500 µg/L	±8%	<120 s	0.983 (ICP-OES)
Online TOC	0–50,000 mg/L	±3%	<180 s	0.943 (GC-FID TPH)

3.5. Operational Efficiency and Resource Consumption

Implementation of the AI + IoT adaptive control system (Condition C) resulted in substantial reductions in resource consumption compared to conventional fixed-parameter operation (Condition A). Aeration energy consumption was reduced by 34.7%, from 2.88 ± 0.14 kWh per cycle to 1.88 ± 0.09 kWh per cycle ($p < 0.001$). Nutrient (NH_4Cl and K_2HPO_4) consumption decreased by 28.5% due to demand-driven dosing based on real-time metabolic activity monitoring. Overall operational cost, calculated based on electricity, nutrients, and consumables, was reduced by 31.2% (Table 5).

Table 5: Resource Consumption and Operational Cost Comparison per 72-Hour Cycle

Parameter	**Condition A (Fixed)**	**Condition C (Adaptive)**	**Reduction (%)**	**p-value**
Aeration Energy (kWh)	2.88 ± 0.14	1.88 ± 0.09	34.7	<0.001
NH_4Cl Consumption (g)	42.6 ± 2.3	30.8 ± 1.7	27.7	<0.001
K_2HPO_4 Consumption (g)	18.4 ± 1.1	12.9 ± 0.8	29.9	<0.001
NaOH Consumption (mL)	124.5 ± 8.2	98.7 ± 6.4	20.7	0.003
Total Operational Cost (USD)[1]	3.82 ± 0.21	2.63 ± 0.15	31.2	<0.001

Data represent mean ± SD of 15 consecutive cycles. [1]Cost calculation based on electricity rate $0.12/kWh and bulk chemical prices. Statistical comparisons by independent t-test.

3.6. Microbial Community Stability and Metagenomic Analysis

16S rRNA gene amplicon sequencing revealed remarkable stability of the synthetic microbial consortium over 45 consecutive operational cycles (approximately 135 days). The relative abundances of the three engineered strains remained within ±5% of their initial inoculation proportions throughout the experimental period (Fig. 4). P. putida KT2440-AB maintained a relative abundance of 43.2–47.8%, R. erythropolis PR4-PAH remained at 28.5–32.4%, and C. metallidurans CH34-HM constituted 23.1–26.7% of the total bacterial community.

Alpha diversity metrics (Shannon index = 1.42 ± 0.18; Simpson index = 0.71 ± 0.08) confirmed low community complexity consistent with a defined synthetic consortium. Beta diversity analysis using Bray-Curtis dissimilarity revealed minimal temporal variation (mean dissimilarity = 0.086 ± 0.023), indicating robust community stability. Detection of low-abundance (<1%) indigenous wastewater bacteria was observed in later cycles, but these did not significantly impact pollutant degradation performance or consortium stability.

3.7. Enzyme Activity Profiles

Enzyme activity measurements throughout the 72-hour treatment cycles revealed distinct temporal patterns corresponding to sequential pollutant degradation (Table 6). Alkane hydroxylase activity in P. putida KT2440-AB peaked at 36 h (3.86 ± 0.24 μmol min^{-1} mg^{-1}), coinciding with the rapid phase of TPH degradation. PAH dioxygenase activity in R. erythropolis PR4-PAH exhibited maximum activity at 48 h (187.4 ± 15.2 nmol min^{-1} mg^{-1}), consistent with the slightly delayed kinetics of PAH catabolism. Pb^{2+} biosorption by C. metallidurans CH34-HM showed sustained activity throughout the 72-hour cycle, reaching maximum capacity (312.6 ± 24.8 mg/g) at the cycle endpoint.

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ISSN (Print): 2321-7510 | ISSN (Online): 2321-7529
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Table 6: Temporal Enzyme Activity Profiles During 72-Hour Bioreactor Cycle (Condition C)

Time (h)	Alkane Hydroxylase (μmol min^{-1} mg^{-1})	PAH Dioxygenase (nmol min^{-1} mg^{-1})	Pb^{2+} Biosorption (mg/g)
0	2.47 ± 0.18	156.8 ± 12.3	287.4 ± 21.6
12	3.12 ± 0.21	162.4 ± 13.5	294.6 ± 22.8
24	3.54 ± 0.26	174.8 ± 14.7	301.2 ± 23.4
36	3.86 ± 0.24	182.6 ± 15.8	306.5 ± 24.1
48	3.21 ± 0.22	187.4 ± 15.2	310.2 ± 24.6
60	2.68 ± 0.19	176.3 ± 14.1	311.8 ± 24.9
72	2.15 ± 0.16	165.7 ± 13.2	312.6 ± 24.8

Data represent mean ± SD of triplicate bioreactor samples collected during cycle 25.

4. DISCUSSION

4.1. Synergistic Degradation by Engineered Synthetic Consortium

The successful construction and validation of a three-strain synthetic microbial consortium capable of simultaneous hydrocarbon, PAH, and heavy metal degradation represents a significant advancement in engineered bioremediation systems. The observed synergistic interactions, wherein the ternary consortium (91.6% TPH degradation) significantly outperformed even the best binary combination (79.8% TPH degradation), underscore the value of rationally designed multi-species communities for addressing complex, multi-component industrial pollutants.

The metabolic complementarity of the engineered strains is likely responsible for the observed synergism. P. putida KT2440-AB initiates rapid alkane oxidation, generating partially oxidized intermediates that may be further metabolized by R. erythropolis PR4-PAH or serve as co-substrates for enhanced PAH dioxygenase expression [25]. Concurrently, C. metallidurans CH34-HM sequesters toxic heavy metals (particularly Pb^{2+}), alleviating metal-induced inhibition of hydrocarbon-degrading enzyme systems in the other consortium members [26]. This division of metabolic labor and cross-protection mechanism is consistent with recent theoretical frameworks for designing robust synthetic microbiomes [27].

The stable maintenance of consortium composition over 45 operational cycles (<5% population drift) is particularly noteworthy and addresses a critical challenge in the field application of synthetic microbial communities. The observed stability may be attributed to several factors: (i) the use of genomically integrated modifications rather than plasmid-borne genes, minimizing fitness costs; (ii) complementary metabolic niches that reduce direct competition; and (iii) the controlled bioreactor environment that minimizes invasion by indigenous microorganisms [28].

4.2. AI-Guided Process Optimization and Predictive Modeling

The RF-BioOpt model demonstrated exceptional predictive accuracy ($R^2 = 0.978$) for pollutant removal efficiency, validating the utility of ensemble machine learning approaches for bioremediation process optimization. The model‘s performance compares favorably with

International Journal of Research and Technology (IJRT)
International Open-Access, Peer-Reviewed, Refereed, Online Journal
ISSN (Print): 2321-7510 | ISSN (Online): 2321-7529
| An ISO 9001:2015 Certified Journal |

previously reported ANN-based bioremediation models (R^2 = 0.92–0.96) [29] and offers the additional advantages of Random Forest algorithms, including robustness to outliers, inherent feature importance quantification, and reduced risk of overfitting.

The identification of C/N ratio and dissolved oxygen as the two most influential process parameters (contributing 18.3% and 15.6% to model predictions, respectively) has direct practical implications for industrial bioremediation operations. These parameters are readily controllable through nutrient dosing and aeration adjustments, making them ideal targets for adaptive process control strategies.

Importantly, the AI-optimized fixed-parameter condition (Condition B) achieved substantially higher pollutant removal (86.7% TPH) compared to conventional operation (71.5% TPH), demonstrating that computational optimization alone—even without adaptive control—can yield significant performance improvements. This finding suggests that AI-based process optimization could be implemented as an initial, lower-cost upgrade to existing industrial bioremediation infrastructure.

4.3. Real-Time Monitoring and Adaptive Control

The integration of IoT biosensor networks with adaptive PLC control represents the most transformative aspect of the intelligent bioremediation platform. The ability to continuously monitor critical process parameters and dynamically adjust operating conditions in response to real-time biological activity fundamentally shifts bioremediation from a passive, open-loop process to an active, closed-loop cyber-biological system [30].

The electrochemical Pb^{2+} biosensor developed for this study addresses a critical analytical gap in bioremediation monitoring. Conventional heavy metal quantification requires offline ICP-OES or AAS analysis with sample preparation delays of hours to days, precluding real-time process control. The DNAzyme-based biosensor, with its 2-minute response time and strong correlation with reference ICP-OES measurements (R^2 = 0.983), enables for the first time the integration of heavy metal removal kinetics into adaptive control algorithms.

The 31.2% reduction in operational costs achieved through adaptive control (Table 5) has substantial economic implications for industrial-scale bioremediation. For a medium-sized wastewater treatment facility processing 10,000 m^3/day, this translates to annual cost savings exceeding $150,000, providing a compelling economic justification for investment in intelligent bioremediation infrastructure.

4.4. Comparison with Previous Studies

Direct comparison of the present results with previous experimental studies confirms the superior performance of the integrated intelligent approach. Conventional bioremediation of petroleum-contaminated wastewater typically achieves TPH removal efficiencies of 50–75% within 7–14 days [31]. The 94.3% TPH degradation achieved in 72 hours under adaptive control represents a >2-fold improvement in both rate and extent of removal.

Recent studies employing single-strain engineered bacteria for specific pollutant classes have reported variable success. For example, engineered E. coli expressing heterologous PETases achieved 45–60% PET nanoparticle degradation over 5 days [32]. The 3D-bioprinted synthetic microbiome described by Wu et al. achieved 78% sulfamethoxazole degradation and 65% PAH removal in 96 hours [33]. The present study's 94.3% TPH and 89.7% PAH removal in 72 hours compares favorably with these benchmarks, while additionally addressing heavy metal co-contamination—a feature not demonstrated in previous studies.

The genetic "expiry-date" circuits described by Kim et al. for biocontainment of engineered bacteria achieved GMO escape rates $<10^{-10}$ [34]. While biocontainment was not a primary focus of the present study, the genomic integration strategy employed for all three engineered strains provides a foundation for future incorporation of such safety mechanisms.

4.5. Limitations and Future Directions

Several limitations of the present study warrant acknowledgment. First, experiments were conducted using simulated industrial wastewater rather than actual industrial effluents. Real-world waste streams contain additional complexity, including surfactants, corrosion inhibitors, and variable pollutant compositions, which may affect consortium performance and sensor reliability. Second, the 45-cycle operational period (135 days) provides evidence of medium-term stability, but longer-term studies (>1 year) are required to assess evolutionary stability and potential loss of engineered functions. Third, the electrochemical Pb^{2+} biosensor, while effective for the target analyte, does not provide multi-metal quantification; development of multiplexed biosensor arrays is a priority for future work.

Future research directions should include: (i) field validation at pilot scale (≥1000 L) using actual industrial wastewater; (ii) incorporation of additional engineered strains targeting emerging contaminants such as PFAS and microplastics; (iii) development of digital twin models enabling predictive simulation and scenario analysis; (iv) integration of biocontainment systems (e.g., kill switches, auxotrophic dependencies) for enhanced biosafety; and (v) techno-economic analysis and life cycle assessment to quantify environmental and economic benefits at industrial scale.

5. CONCLUSION

This study successfully developed and experimentally validated an integrated intelligent bioremediation platform that combines AI-guided synthetic microbial consortia, IoT-enabled biosensor networks, and adaptive process control—realizing the core principles of Industry 5.0 in environmental biotechnology. The engineered three-strain synthetic consortium achieved 94.3% TPH degradation, 89.7% PAH removal, and 82.5% Pb^{2+} biosorption within 72 hours under AI-optimized adaptive control, significantly outperforming conventional fixed-parameter bioremediation. Real-time IoT monitoring enabled dynamic process optimization that reduced operational costs by 31.2% while maintaining robust microbial community stability over 45 operational cycles. These findings demonstrate that intelligent cyber-biological systems represent a transformative approach to industrial bioremediation, offering a scalable, sustainable, and economically viable solution for addressing complex environmental pollution challenges in the Industry 5.0 era.

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