

Article

Bioelectrochemical Treatment of Ibuprofen-Contaminated Wastewater Using Hypersaline Sediment Microbiomes: Emergence of *Chromohalobacter* as a Key Degradator

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Abstract

Ibuprofen (IBU), a widely used anti-inflammatory drug, is increasingly found in aquatic environments, particularly in pharmaceutical wastewater, raising concerns about its persistence and ecological risks. This study investigates IBU removal using a bioelectrochemical system (BES) under laboratory conditions with synthetic pharmaceutical wastewater (SPWW) and halophilic microbiomes from two hypersaline sediments of Chott El-Djerid (CJ-S1 and CJ-S2). Three bioanode reactors were operated at +0.1 V/SCE for 15 days with IBU concentrations of 60 and 150 ppm, with or without glucose (Glc) (20 ppm) as a co-substrate. IBU degradation was confirmed by LC-MS, COD removal, and FTIR analyses, showing removal efficiencies approaching 100%. Electrochemical performance varied with the inoculum: CJ-S2 produced a higher current density ($38.02 \pm 0.15 \text{ mA m}^{-2}$) than CJ-S1. Metataxonomic analysis revealed strong enrichment of *Chromohalobacter* spp., while the combination of CJ-S2, 60 ppm IBU, and Glc promoted a more diverse consortium dominated by *Halomonas* spp. and *Bacillus shackletonii*. These findings highlight that microbial origin and co-substrate availability critically shape bacterial community structure, electroactivity, and degradation efficiency. The CJ-S2 microbiome is a promising candidate for developing robust electroactive bioanodes for treating pharmaceutical wastewater.



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1. Introduction

Pharmaceuticals and personal care products (PPCPs) are continuously released into aquatic environments through municipal wastewater treatment plant effluents, industrial discharges, hospital wastewater, and pharmaceutical manufacturing activities [1,2]. Due to their specific biological activity, persistence, and incomplete removal by conventional treatment methods, a wide range of PPCPs has been detected in surface water, groundwater and even drinking water at concentrations ranging from ng L^{-1} to mg L^{-1} [3–5].

These uncontrolled discharges of bioactive compounds and their transformation products may potentially cause serious health problems for humans, aquatic organisms, and ecosystem functioning [6,7].

Among PPCPs, non-steroidal anti-inflammatory drugs (NSAIDs) are the most prescribed pharmaceuticals worldwide [8]. Ibuprofen (IBU) [2-(4-Isobutylphenyl) propanoic acid, IBU] is the third-most consumed drug [9]. Due to its extensive use and partial removal during wastewater treatment processes, IBU has been frequently detected in aquatic environments and wastewater streams. Reported concentrations span multiple orders of magnitude, ranging from tens of ng L^{-1} in surface waters to $\mu\text{g L}^{-1}$ levels in municipal wastewater influents and effluents [10].

Beyond its widespread occurrence, IBU has been shown to exert toxic effects on aquatic species even at low concentrations. According to Menicagli et al. [11], exposing *Cymodocea nodosa* to concentrations (0.25–2.2–25 $\mu\text{g L}^{-1}$) of IBU for 12 days induced oxidative stress and damaged the photosynthetic mechanism, particularly at the highest concentration. Chronic exposure has further been linked to reproductive impairment, oxidative imbalance, and genotoxic effects in various aquatic organisms [12]. This wide concentration range highlights the persistence of IBU in the environment and emphasizes the need for treatment strategies capable of addressing both diluted municipal effluents and more concentrated point-source discharges.

IBU is typically detected at low concentrations ($\text{ng}–\mu\text{g L}^{-1}$) in municipal wastewater influents and effluents but can reach much higher levels up to 400 ppm in pharmaceutical manufacturing effluents reported in Iran [13]. Accordingly, the IBU concentrations investigated in this study were selected to represent pharmaceutical point-source discharges, enabling the assessment of the operational robustness and degradation capacity of bioelectrochemical systems (BESs) under realistic worst-case conditions.

Various treatment technologies have been investigated for the removal of pharmaceuticals and other persistent organic pollutants from wastewater, including chemical, physical, and biological approaches. However, many of these methods remain limited by high operational costs, energy demand, extensive chemical consumption, and limited efficiency toward recalcitrant compounds [14,15]. Consequently, the development of effective and environmentally sustainable treatment strategies remains a major challenge.

In this context, BESs have emerged as a promising approach for the removal of recalcitrant organic pollutants and the activity of electroactive microbial biofilms [16]. In BESs, microorganisms catalyze redox reactions at electrode surfaces, enabling the oxidation or reduction in contaminants by using solid electrodes as terminal electron acceptors or donors. This approach reduces the need for intensive aeration or chemical additives, potentially lowering energy and chemical inputs compared to conventional treatment technologies [17,18]. Recently, BESs have received more attention for the removal of IBU. Wang et al. [19] reported that electrostimulated biofilms significantly enhanced IBU transformation, involving electroactive microbial consortia such as *Geobacter* and *Desulfovibrio*. In addition, BESs have already been applied for IBU degradation in different configurations. For example, Youssef et al. [20] used a CW-MFC system for the degradation of 10 ppm of IBU, achieving an elimination efficiency of 62%, while Qin et al. [21], using the same system at an initial concentration of 5 ppm, reported a removal efficiency of 89.29%. Similarly, Xu et al. [22] investigated a microbial fuel cell (MFC) operated at an initial concentration of 10 ppm and obtained an IBU removal efficiency of 75.94%. These studies highlight the key role of electroactive biofilms and electron transfer processes in IBU biotransformation. However, despite these promising performances, most reported BES-based studies were conducted under conventional operating conditions, with moderate salinity and relatively low pollutant concentrations, leaving the performance of BESs under high concentrations

more representative of real wastewater, which is still insufficiently explored. In particular, further investigation is needed at elevated salinity levels, typically ranging from 22,168 to 7651 mg L⁻¹, relevant to pharmaceutical wastewater [23–25], where the application of halophilic consortia could play a key role in maintaining system performance and stability.

In this regard, the use of electroactive halophilic microbial communities from hypersaline environments represents a novel strategy for improving the robustness and efficiency of BESs for the treatment of recalcitrant pharmaceutical pollutants [26]. Hypersaline environments constitute unique ecological niches hosting highly adapted, electroactive and metabolically versatile microbial communities, capable of surviving under high pollutant loads [27]. Such environments represent valuable reservoirs of resilient exoelectrogenic microorganisms with a strong potential for bioelectrochemical applications [28]. The Tunisian hypersaline sediments of Chott El Dejerid (THSCJ) have been identified as a rich source of halophilic electroactive bacteria, previously shown to support efficient bioanode formation and stable current generation under hypersaline conditions. Bioanodes enriched from these sediments achieved high degradation efficiencies for refractory pollutants such as azo dyes in real wastewater [29,30], and fungicides in synthetic wastewater [31]. However, the potential of these hypersaline microbiomes for the bioelectrochemical removal of pharmaceuticals contaminants, such as IBU, remains largely unexplored.

Hence, the present study aims to evaluate the biofilm formation, electrochemical performance, and IBU removal efficiency of halotolerant bacterial bioanodes in laboratory-scale BES inoculated with different hypersaline microbiomes from THSCJ. This work seeks to assess the potential of these resilient bacterial communities for the treatment of higher pharmaceutical concentrations representative of point-source discharges. We hypothesize that under saline conditions, IBU degradation and electrochemical performance in BES would be improved.

2. Materials and Methods

2.1. Collection of Hypersaline Sediment Samples

Two hypersaline sediment samples, designated CJ-S1 and CJ-S2, were collected from Chott El-Djerid, located in southern Tunisia during July 2021 and May 2022, respectively. Samples CJ-S1 (N: 33°57'272"; E: 008°24'393") and CJ-S2 (N: 33°59'965"; E: 008°25'332") were stored in sterile bags at +4 °C until utilization. Physicochemical parameters of the sediment samples were analyzed as reported by Saidi et al. [27]. Specifically, pH and electrical conductivity were measured electrochemically, total organic carbon and orthophosphate (PO₄³⁻) were quantified by colorimetry, bicarbonate (HCO₃³⁻) and chloride (Cl⁻) were analyzed by titrimetry, sulfate (SO₄²⁻) was determined by spectrometry, and major cations such as (Ca²⁺), (K⁺), (Mg²⁺), (Na⁺), and (Fe²⁺) were measured by inductively coupled plasma atomic emission spectroscopy ICP.

2.2. Ibuprofen

Ibuprofen was used in this study from different sources, and the form of the commercial pharmaceutical product Ibuphil (600 mg, SIMED laboratory, Sfax, Tunisia) was used for experiments. The tablets were crushed and dissolved in dimethyl sulfoxide (Pallav Chemicals & solvents Pvt. Ltd., Mumbai, India) to prepare a stock solution of IBU at a concentration of 30,000 ppm which was subsequently diluted to achieve final concentrations of 60 or 150 ppm in the reactors. In addition, analytical grade IBU (≥98% purity, Toronto Research Chemicals, Toronto, ON, Canada) was used as standard.

2.3. Synthetic Pharmaceutical Wastewater Composition

All experiments were performed in duplicate in minimal salt medium supplemented with IBU as the target substrate and glucose (Glc) as co-substrate. The detailed composition of the minimal salt medium (MSM) was as follows (per liter): 150 g NaCl, 3 g K₂HPO₄, 1 g NH₄Cl, 2 g MgCl₂, 0.5 g MgSO₄, and 3.7 g Na₂HPO₄ (Loba Chemie Pvt. Ltd., Mumbai, India). IBU was added to MSM at concentrations of 60 or 150 ppm to the reactors simultaneously with 20 ppm Glc to determine its effect on IBU removal and current generation. The pH was adjusted to 7.2 and the experiments were carried out at a temperature of 25 ± 2 °C.

2.4. Reactor Configuration and Electrochemical Protocols

In this article, the degradation of IBU in synthetic water streams was studied using a laboratory-scale experimental apparatus. All experiments were conducted in a 500 mL glass three-electrode reactor containing a mixture of 70% synthetic pharmaceutical wastewater and 30% hypersaline sediment, expressed as a volume/volume (*v/v*) ratio.

The hypersaline sediments and synthetic wastewater were thoroughly mixed by vigorous magnetic stirring until a homogeneous suspension was obtained. After the homogenization step, the reactors were hermetically sealed. Biological degradation controls were incubated without polarization.

For the bioelectrochemical experiments, a 3D carbon felt as a standard electrode (1 × 2 × 3 cm, Mersen, Courbevoie, France) was used as the working electrode (WE), a platinum grid (4 cm × 3 cm, Heraeus, Hanau, Germany) was used as the auxiliary electrode (AE), and a saturated calomel electrode (SCE) was placed between WE and AE as the reference electrode. All three electrodes were connected to a Multichannel Potentiostat/Galvanostat (OGF 500, Orignalys, Rillieux-la-Pape, France) managed by the OrigaMaster software (version 2.5.0.6). Polarization at +0.1 V vs. SCE was applied to the WE [27]. Chronoamperometric (CA) current densities of bacterial bioanodes were determined as described in previous studies [27]. Moreover, the cyclic voltammetry (CV) measurements were conducted in situ after stopping CA, within a potential range of −1 to +0.7 V/SCE, at a scan rate of 1 mV s^{−1}. Additionally, a bioelectrochemical control experiment was performed using only sediment (without IBU and Glc) under the same electrochemical conditions, to evaluate the current production derived from organic matter in the sediment.

In parallel, biotic control experiments were performed using active bacterial inoculum only to assess the natural biological capacity of microorganisms to degrade IBU without electrochemical stimulation, as shown in Table 1. Abiotic controls were conducted using sterilized setup with polarized carbon felt under the same applied potential to verify the contribution of purely electrochemical IBU oxidation.

Table 1. Summary of bioelectrochemical setups, treatment conditions, and corresponding abiotic and biotic controls.

	Code	[IBU] (ppm)	[Glc] (ppm)	Sediment Type
Experiments	60IBU/Glc-S1	60	20	CJ-S1
	60IBU-S1		0	
	60IBU/Glc-S2		20	CJ-S2
	60IBU-S2		0	

Table 1. Cont.

	Code	[IBU] (ppm)	[Glc] (ppm)	Sediment Type
Experiments	150IBU/Glc-S1	150	20	CJ-S1
	150IBU-S1		0	
	150IBU/Glc-S2		20	CJ-S2
	150IBU-S2		0	
Abiotic controls	60IBU/Glc-S1	60	20	Without sediments
	60IBU-S1		0	
	60IBU/Glc-S2		20	
	60IBU-S2		0	
	150IBU/Glc-S1	150	20	
	150IBU-S1		0	
	150IBU/Glc-S2		20	
	150IBU-S2		0	
Biotic controls	60IBU/Glc-S1	60	20	CJ-S1
	60IBU-S1		0	
	60IBU/Glc-S2		20	CJ-S2
	60IBU-S2		0	
	150IBU/Glc-S1	150	20	CJ-S1
	150IBU-S1		0	
	150IBU/Glc-S2		20	CJ-S2
	150IBU-S2		0	

2.5. Anodic Biofilm Study

At the end of experiments, bioanodes were removed from reactors and immediately treated with 0.01% acridine orange for 10 min to examine the biofilm structure. Following incubation, the bioanodes coupons were carefully washed with sterile physiological water (0.9% NaCl) and left to dry in the dark at room temperature [17]. Biofilms were then imaged with epifluorescence microscopy (Dm 4000B, Leica, Wetzlar, Germany).

2.6. Analytical Methods

2.6.1. Chemical Oxygen Demand Analysis

Before and after bioelectrochemical and biological control treatments, the chemical oxygen demand (COD) was measured to assess the organic load in all samples. The LCW 925 kit (Hach, Loveland, CO, USA) was used to remove chloride ions then COD was determined using the LCK 514 kit (Hach, Loveland, CO, USA) following the manufacturer's instructions. Absorbance measurements were conducted using UV-Visible spectrophotometer (DR 3900, Hash, Loveland, CO, USA) at 600 nm. The COD removal efficiency was calculated using the following equation:

$$\text{COD removal efficiency (\%)} = \frac{\text{COD initial} - \text{COD final}}{\text{COD initial}} \times 100$$

The coulombic efficiency was calculated based on changes in chemical oxygen demand (COD) concentration using the equation below:

$$CE = \frac{\int_0^t Idt}{FbV\Delta COD} \times 100$$

where I is current density, t is the time, F is the Faraday constant ($96,485 \text{ C}\cdot\text{mol}^{-1}$), b is the number of electrons exchanged per mole of O_2 , V is the reactor volume, and ΔCOD is the change in substrate concentration over time.

2.6.2. Liquid Chromatography–Mass Spectrometry (LC–MS) Analysis

Liquid–liquid extraction is performed using dichloromethane as a solvent, following the protocol described by [27]. Briefly, 5 mL of the electrolyte was mixed with 5 mL of solvent, and a $\text{NaCl}/\text{MgSO}_4$ was added in solid form in a 1:1 ratio to promote phase separation. After vortexing and separation (by settling and centrifugation), a second extraction was performed to improve recovery. The lower organic phases were collected, pooled, and evaporated at room temperature. The resulting dry residue was reconstituted in a (1:1) methanol/water solution, filtered, and transferred into an LC-MS vial for examination. All quantitative analyses were performed on an Agilent 1290 series liquid chromatography system coupled to an Agilent 6460 triple quadrupole mass spectrometer (Agilent, Santa Clara, CA, USA) with Agilent Jet Stream electrospray ionization (AJS-ESI) technology. Liquid chromatographic separation was performed on an Eclipse XDB-C18 column ($3.0 \times 150 \text{ mm}$, $3.5 \mu\text{m}$, Agilent, Santa Clara, CA, USA). Optimal separation was achieved using water (A) and acetonitrile (B), both containing 0.1% formic acid, as mobile phases. Linear gradient elution was applied starting with 20% B and increased to 60% over 10 min and held at this percentage for 6 min. A constant flow rate of $0.3 \text{ mL}/\text{min}$ was used with a sample injection volume of $15 \mu\text{L}$. The column oven was kept at a constant temperature of 30°C for the duration of the analysis. Parameters for MS/MS operation were as follows: The drying gas temperature was set at 300°C at a flow of $12 \text{ L}/\text{min}$ and the sheath gas temperature was set at 300°C at a flow of $11 \text{ L}/\text{min}$. Nitrogen was used as drying gas and argon as collision gas. The capillary voltage and nozzle voltage were set at 4000 and 500 V, respectively, and the nebulizer pressure used was 35 psi. The MRM mode was used with a mass transfer of the precursor ion 205 (Q1) to the product ion 161 (Q3). A collision energy of five was applied.

2.6.3. Fourier Transform Infrared (FTIR) Spectroscopy Analysis

Possible modifications of functional groups of IBU were analyzed by FTIR after its bioelectrochemical degradation under ambient conditions in transmission mode. Measurements were performed in the range of $4000\text{--}400 \text{ cm}^{-1}$ using an FTIR apparatus (PerkinElmer model 1720-X, PerkinElmer, Waltham, MA, USA). A non-degraded IBU solution was used as a control.

2.6.4. Bacterial Community Analysis

After 15 days of operation, a culture-independent approach was applied to study the bacterial community occurring both in the original sediments CJ-S1 and CJ-S2, in the anode biofilm and in the anolyte, for the following reactors, namely 150IBU-S2, 150IBU/Glc-S2, 60IBU/Glc-S1 and 60IBU/Glc-S2, as mentioned in Table 1. Genomic DNA extracted from different samples was obtained using Invitrogen Soil kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. To verify the purities, qualities, and the concentrations (ng L^{-1}), the DNA samples were evaluated using a micro volume Nanodrop with an absorbance ratio (A_{260}/A_{280}). Subsequently, Illumina Miseq 16S rRNA

sequencing was performed to analyze bacterial community composition. The 16S rRNA gene V3-V4 variable region PCR primers 515F/806R were used in a single-step 30-cycle PCR using the HotStarTaqPlus Master Mix Kit (Qiagen, Germantown, MD, USA). The bacterial community composition was further analyzed using RStudio (version 4.4.1) with appropriate packages for ecology.

Representative 16S rRNA gene sequences were taxonomically assigned using BLASTn (version 2.16.0) against the NCBI 16S rRNA reference database. Data analysis and visualization were performed using RStudio version 4.4.1 [32]. The vegan package (version 2.6.4) was used to calculate the Shannon diversity index, phyloseq (version 1.42.0) [33] and pheatmap (version 1.0.13) [34] for bacterial community data handling and heat map construction, and UpSetR (version 1.4.0) [35] for UpSet plot analysis.

3. Results

3.1. Physicochemical Characteristics of THSCJ

The chemical and physical properties of the two hypersaline sediments are summarized in Table 2. Physicochemical analyses showed that CJ-S2 had significantly higher conductivity (74.1 mS cm^{-1}) compared to CJ-S1 (30.7 mS cm^{-1}). This high conductivity facilitates ion transport within the reactors, thereby improving extracellular electron transfer from electroactive biofilms, which enhances BES performance. In addition, CJ-S2 contained higher concentrations of ions, particularly chloride, magnesium, potassium, and iron at concentrations of 130, 32.4, 71.6, and $18.6 \text{ (g kg}^{-1} \text{ dry matter (DM))}$, respectively, compared to CJ-S1. Orthophosphate was also more abundant in CJ-S2 ($10.19 \text{ of PO}_4^{3-} \text{ mg kg}^{-1} \text{ DM}$). These ions play fundamental roles in bacterial metabolism, contributing to cell growth, metabolic processes, and respiratory activities, and can also function as potential electron acceptors in BES.

Table 2. Physicochemical characteristics of CJ-S1 and CJ-S2.

Essai(s)	CJ-S1	CJ-S2
Conductivity (mS cm^{-1})	30.7	74.1
Total organic carbon ($\text{g kg}^{-1} \text{ DM}^*$)	58.8	19.9
Orthophosphate ($\text{mg kg}^{-1} \text{ DM}$)	1.289	10.19
Chloride ($\text{g kg}^{-1} \text{ DM}$)	17.7	130
Sulfate ($\text{g kg}^{-1} \text{ DM}$)	16.3	9.29
Calcium ($\text{g kg}^{-1} \text{ DM}$)	196.3	97.8
Magnesium ($\text{g kg}^{-1} \text{ DM}$)	9.53	32.4
Potassium ($\text{g kg}^{-1} \text{ DM}$)	3.21	71.6
Sodium ($\text{g kg}^{-1} \text{ DM}$)	35.16	12.9
Iron ($\text{g kg}^{-1} \text{ DM}$)	4.58	18.6

DM: dry matter *.

3.2. Bioanode Growth and Bioelectrochemical Characterization

All experiments were conducted in duplicate, and the results were based on averages across replicates.

Both THSCJ were compared as sources of halophilic bacteria for (i) bioelectrocatalytic anode formation, and (ii) IBU degradation at two concentrations of 60 and 150 ppm. In all experiments, IBU was used as the primary substrate, while Glc served as co-substrate. Two replicates of each BES were used simultaneously, and the current densities shown in Figure 1 represent the average values obtained. Overall, sixteen bioelectrochemical

experiments were performed with synthetic pharmaceutical wastewater (SPWW). The working electrodes were polarized at +0.1 V for 15 days to allow the formation of mature bioanodes on carbon-based electrodes colonized by halo-exoelectrogenic bacteria [15]. Current production was observed after almost two days of polarization for all bioanodes inoculated with sediments CJ-S1 or CJ-S2 (Figure 1).

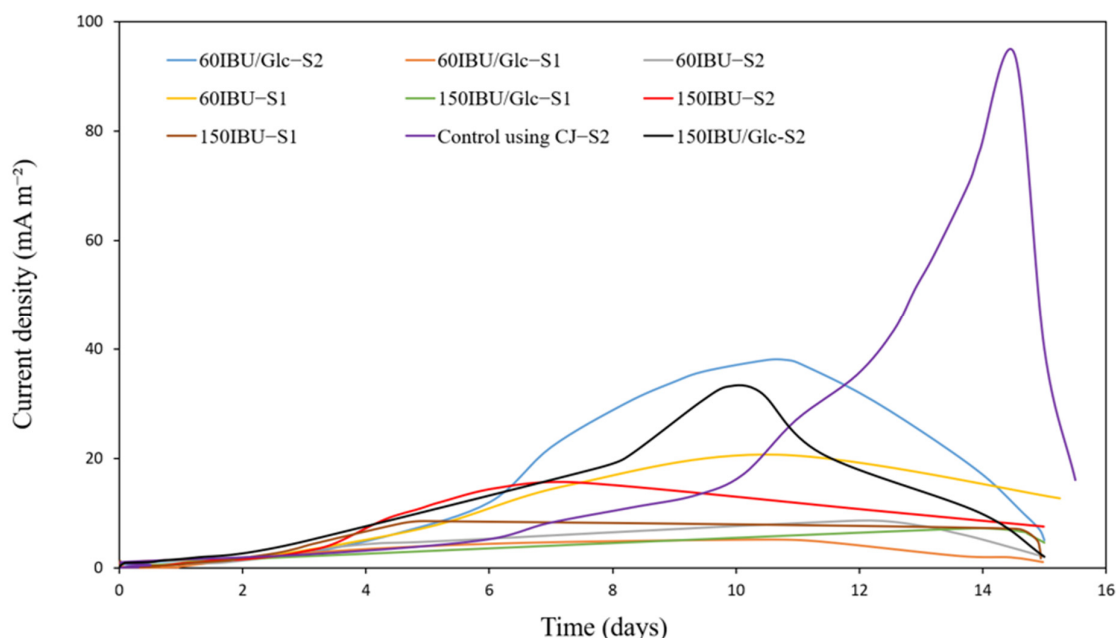


Figure 1. Evolution of the current density (A m^{-2}) versus time (day) using IBU and Glc as electron donor on a carbon felt electrode inoculated with CJ-S1 or CJ-S2.

The IBU degradation rates, coulombic efficiency, maximum current densities, retention times, and COD determined under various experimental conditions are summarized in Table 3. The CJ-S2 inoculum showed more electrocatalytic activity than CJ-S1, mainly in terms of current densities. As shown in Figure 1 and Table 3, the bioelectrochemical control experiment conducted using only sediment CJ-S2 produced a current density of $93.9 \pm 0.03 \text{ mA m}^{-2}$, which was three times higher than that recorded when using IBU as the carbon source. These results can be attributed to the potential inhibitory effect of IBU on electroactive microorganisms. Changes in current densities (Figure 1) reflect biofilm formation on the anode surface and correspond to the dynamics of bacterial activity influenced by the inoculum type, IBU concentration and the presence of co-substrate. In fact, the halophilic inoculum from CJ-S2 generated a maximum current density of $38.02 \pm 0.15 \text{ mA m}^{-2}$ when treating 60 ppm IBU and 20 ppm Glc. Similarly, at 150 ppm IBU with 20 ppm Glc, a comparable current density of $33.12 \pm 0.11 \text{ mA m}^{-2}$ was recorded. However, with CJ-S1, the maximum current density reached only $5.15 \pm 0.03 \text{ mA m}^{-2}$ under the same conditions (60 ppm IBU and 20 ppm Glc).

To track the evolution of the bioelectrocatalytic activity of the bioanodes, low-scan-rate (1 mV s^{-1}) cyclic voltammetry was recorded at the beginning and at the end of each experiment. As shown in Supplementary Data Figure S1, the initial cyclic voltammograms exhibited no evidence of electrochemical activity on the carbon felt electrode and no electroactive biofilm, as indicated by the featureless voltametric profiles over the potential range from -0.2 to $+0.7 \text{ V/SCE}$. Interestingly, from -0.2 to -1 , a sharp decrease in the current was observed due to the electrochemical reduction in some compounds in the minimum medium. After achieving the maximum current density, other CVs were performed for all reactors. The cyclic voltammogram of reactor A, which exhibited the maximum current density of $38.02 \pm 0.15 \text{ mA m}^{-2}$, did not show a significant variation compared to

the initial voltammogram. This behavior can be attributed to the limited biofilm biomass on the bioanode surface, despite the presence of a well-developed and electroactive bacterial community ensuring efficient electron transfer. In reactors B, D, E and F, no significant changes were observed in the final cyclic voltammograms compared to initial profiles, particularly within the -0.2 to $+0.7$ V/SCE potential range. In contrast, reactors G and H displayed distinct oxidation and reduction peaks, which were clearly identified, indicating the establishment of an electroactive microbial biofilm on the carbon felt surface.

Table 3. Ibuprofen degradation and removal efficiencies under bioelectrochemical and biological conditions.

Samples	Retention Time	Bioelectrochemical Experiments					Biological Control	
		C _{initial} [IBU] (ppm)	C _{final} [IBU] (ppm)	% IBU Degradation	CE %	Maximum Current Density (mA m ⁻²)	% COD Removal	% COD Removal
60IBU/Glc-S1	11.87	60	0.01 ± 0.04	99.97 ± 0.03	6.54 ± 0.02	5.15 ± 0.03	95.55 ± 0.03	38.07 ± 0.03
60IBU-S1	11.80		0.01 ± 0.05	99.98 ± 0.02	19.86 ± 0.3	20.59 ± 0.08	93.37 ± 0.01	26.80 ± 0.2
60IBU/Glc-S2	11.85		4.26 ± 0.02	92.90 ± 0.05	27.59 ± 0.14	38.02 ± 0.15	93.46 ± 0.01	ND *
60IBU-S2	11.85		28.92 ± 0.11	51.79 ± 0.12	9.06 ± 0.02	8.59 ± 0.09	56.79 ± 0.3	ND
150IBU/Glc-S1	11.87	150	28.74 ± 0.09	80.84 ± 0.01	8.36 ± 0.1	7.16 ± 0.05	95.22 ± 0.03	31.37 ± 0.3
150IBU-S1	11.85		79.96 ± 0.11	46.69 ± 0.07	7.03 ± 0.01	8.45 ± 0.06	56.13 ± 0.2	13.43 ± 0.3
150IBU/Glc-S2	11.86		1.175 ± 0.02	99.22 ± 0.08	25.46 ± 0.16	33.13 ± 0.11	79.16 ± 0.12	ND
150IBU-S2	11.87		36.62 ± 0.04	75.58 ± 0.10	16.59 ± 0.3	15.56 ± 0.06	54.71 ± 0.1	ND

ND *: Not Determined.

The coulombic efficiencies (CE%) recorded in the bioelectrochemical experiments were determined assuming the complete oxidation of both Glc and IBU, which ranged from 6.54 ± 0.02 to 27.59 ± 0.14 , and were consistently lower than the values theoretically expected from Glc oxidation. Although nearly complete IBU degradation was achieved under the 150IBU/Glc-S2 condition, the comparatively lower COD removal indicates that IBU transformation did not result in complete mineralization. The residual COD is likely attributable to the formation and persistence of intermediate organic compounds generated during the degradation process. Control experiments were performed to evaluate non-biological contributions to IBU degradation. In fact, no current generation was observed under abiotic and open-circuit conditions.

The faradaic efficiency was calculated based on COD removal rather than IBU degradation, as COD reflects the overall oxidation of biodegradable organic matter present in reactors. Moreover, IBU degradation does not necessarily imply complete mineralization, and the formation of transformation products prevents an accurate electron balance based solely on IBU removal. Therefore, the calculated Faradic efficiency should be considered as an estimation of the overall electron recovery from oxidizable organic matter than a direct indicator of IBU removal.

3.3. Microscopy Analysis of Biofilm Structure

At the end of the experiments, colonization of the carbon felt electrodes was evaluated, exclusively on the felts' surface. Figure 2 presents the results obtained using an epifluorescence microscope after labeling the electrodes with 0.01% acridine orange. This staining enables the visualization of bacterial cell density and extracellular polymeric substances forming nucleic acid networks on the electrode surface. The epifluorescence images are interpreted based on the fraction of the surface area of two zones: (i) high fluorescence intensity areas, corresponding to significant colonization by bacterial cells and biofilm matrix, and (ii) dark zones, which correspond to an uncolonized surface region. In Figure 2, the panels illustrate the electrode surfaces corresponding to the following BES conditions: Figure 2A-60IBU-S1, Figure 2B-

150IBU/Glc-S1 and Figure 2C 60IBU/Glc-S2. As shown in Figure 2A,B, bioanodes displayed visible colonization while producing lower current densities of 20.59 and 7.16, mA m^{-2} , respectively. Biofilm from 60IBU-S1 BES (Figure 2A) presents a continuous, homogeneous and dense aspect settled between the surface fibers while biofilm from 150IBU/Glc-S1 BES (Figure 2B) showed a non-homogeneous colonization on the carbon fibers with a lower density characteristic. This observation could be attributed to the higher IBU concentration, which may have affected biofilm structure and density. In contrast, Figure 2C, corresponding to the biofilm from 60IBU/Glc-S2 BES, which produced the highest current density ($38.02 \pm 0.15 \text{ mA m}^{-2}$), showed a slightly colonized surface. The biofilm on this electrode appeared sporadic, non-continuous, and composed of sparse clusters of low-density bacterial colonies, attached to only a few fibers on the carbon felt surface.

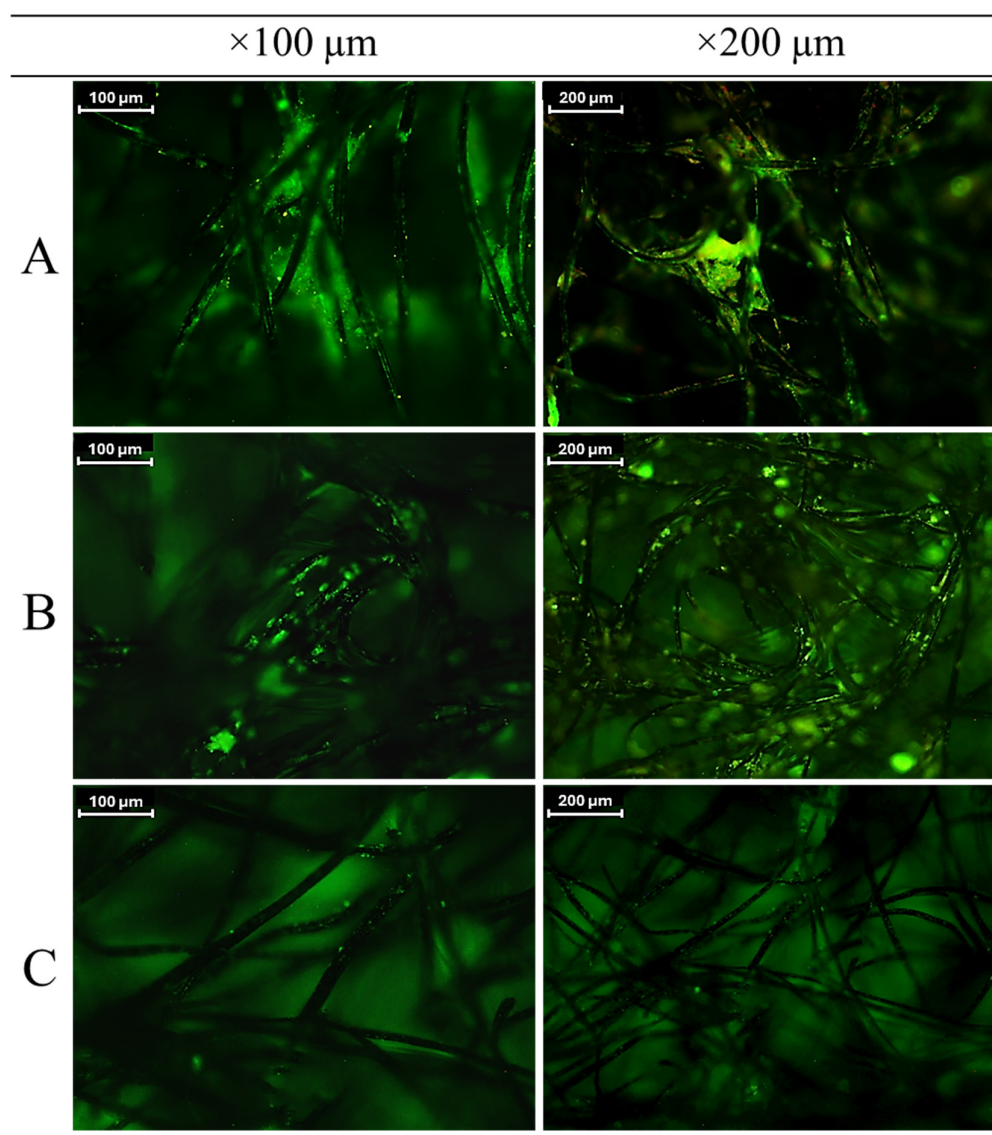


Figure 2. Assessment of bacterial colonization on the carbon felt electrode surfaces using epifluorescence microscopy equipped with two objectives (100 and 200 μm): (A) 60IBU-S1, (B) 150IBU/Glc-S1, (C) 60IBU/Glc-S2.

3.4. FTIR Analysis

At the end of the experiments, electrolytes from different reactors were analyzed by FTIR within the range of $4000\text{--}400 \text{ cm}^{-1}$ to identify possible structural changes in IBU following bioelectrochemical treatment. IBU ($\text{C}_{13}\text{H}_{18}\text{O}_2$) composed of a propanoic acid

group ($-\text{CH}_3-\text{CH}(\text{CH}_3)-\text{COOH}$), an isobutyl group ($-\text{CH}_2-\text{CH}(\text{CH}_3)_2$) and a phenyl ring (C_6H_5-). A spectrum of the untreated IBU control in the solution was used as a reference. As shown in Figure 3, the standard IBU spectrum shows the main characteristic peaks in the fingerprint region ($1500-500\text{ cm}^{-1}$). A large band at $3013-2800\text{ cm}^{-1}$ corresponds to O-H and C-H stretching vibrations in the benzene ring, and a strong absorption at 1715 cm^{-1} characterizes C=O stretching of the carboxylic group ($-\text{COOH}$). The peak at 1416 cm^{-1} represents C-H bending in the aromatic ring. The peak observed at 1323 cm^{-1} is attributed to $-\text{CH}_3$ stretching vibration, while those at 1229 and 1184 cm^{-1} may correspond to C-O and C-O-C stretching of IBU ring groups. Additional peaks are detected at 865 cm^{-1} and 780 cm^{-1} which correspond to C=C bending bands in the aromatic ring. Finally, the region between 1508 and 520 cm^{-1} reflect the isobutyl chain's flexibility and the existence of a monosubstituted aromatic ring.

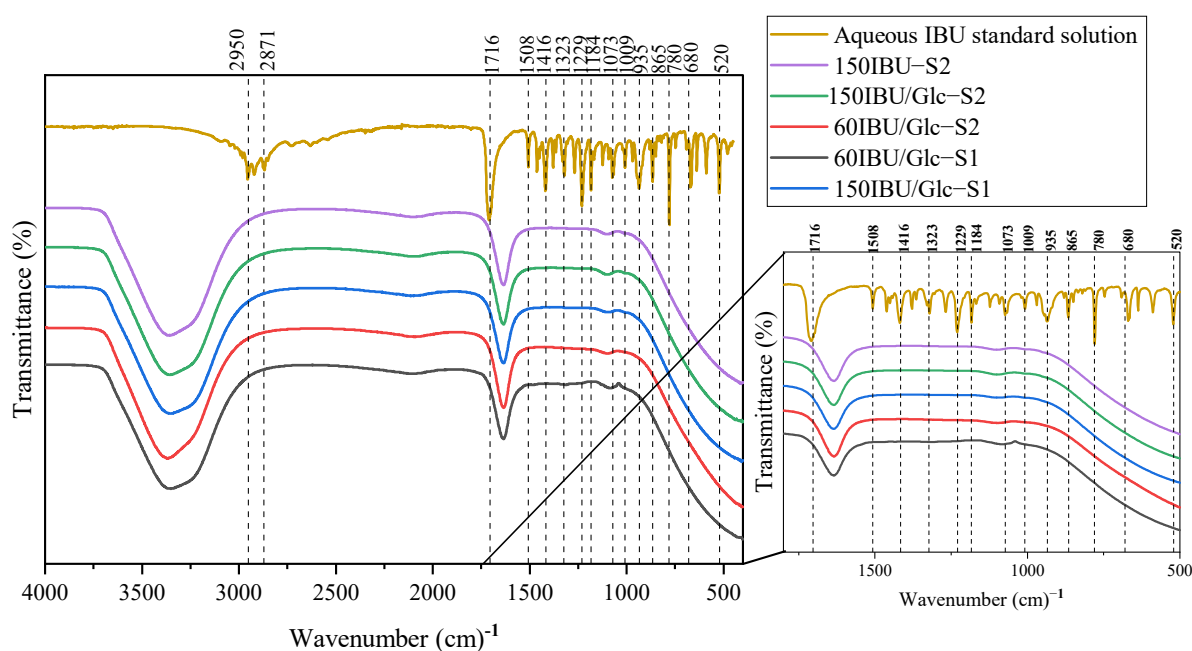


Figure 3. Fourier transform infrared spectra of IBU before and after bioelectrochemical experiments using hypersaline sediments (CJ-S1 or CJ-S2) as inocula.

After bioelectrochemical treatment, significant changes in the FTIR spectra were observed, including a reduction in the number of detectable peaks from 10 to two (Figure 3), indicating major structural transformations of IBU molecules. Characteristic peaks such as the C-O stretching band at 1229 and 1184 cm^{-1} decreased in intensity, while the C=O stretching band at 1715 cm^{-1} partially disappeared indicating the cleavage of the carboxylic and ether bonds in IBU. Moreover, the disappearance of peaks in the fingerprint region ($1500-500\text{ cm}^{-1}$) indicates the breakdown in the aromatic ring and the transformation of IBU. These changes are correlated with the results obtained from LC-MS and COD analyses and confirmed the structural alteration and bioelectrodegradation of IBU during the BES process.

3.5. Liquid Chromatography–Mass Spectrometry (LC–MS) Analysis and Carbon Oxygen Demand (COD) Measurements

IBU degradation was monitored by LC-MS in all bioelectrochemical reactors. COD measurements were performed for bioelectrochemical experiments inoculated with CJ-S1 and CJ-S2 sediments, as well as biological controls inoculated with CJ-S1.

LC-MS analysis revealed a decrease in the IBU peak area at a retention time of approximately 12 min (Figure 4), indicating substantial IBU removal from SPWW. Quantitative analysis confirmed significant degradation and a corresponding reduction in COD (Table 3).

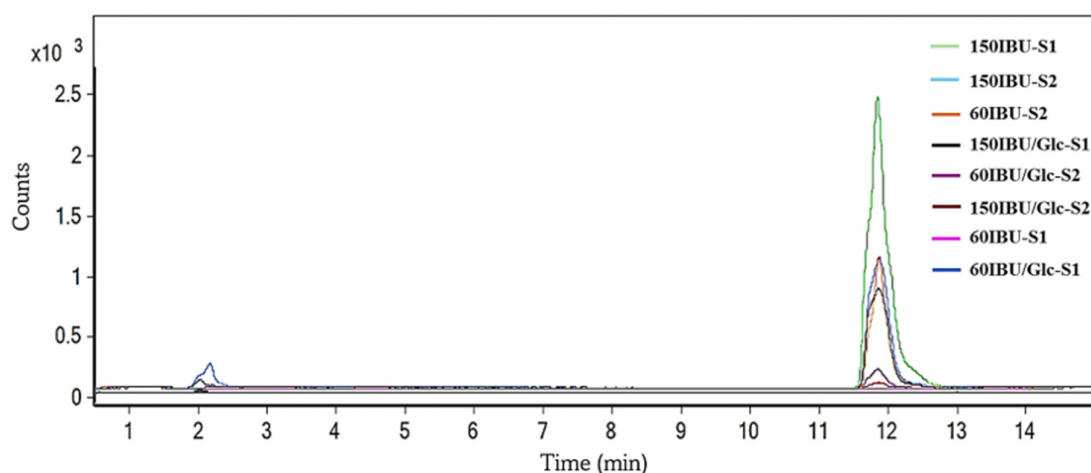


Figure 4. Assessment of IBU degradation using LC-MS analysis of BES electrolytes.

For experiments inoculated with CJ-S1 and 60 ppm IBU, degradation efficiencies reached 99.97 ± 0.03 and $99.98 \pm 0.02\%$, with COD removal of 95.55 ± 0.03 and $93.37 \pm 0.01\%$ for 60IBU/Glc-S1 and 60IBU-S1, respectively. In contrast, biological control experiments showed only 26–38% COD removal. Using CJ-S2, IBU degradation efficiencies ranged from $51.79 \pm 0.12\%$ without Glc (60IBU-S2, Table 3) to $92.90 \pm 0.05\%$ with Glc (60IBU/Glc-S2, Table 3), consistent with COD removal rates of 56.79 ± 0.3 and $93.46 \pm 0.01\%$, respectively. At 150 ppm, both sediments showed clear IBU degradation patterns, with CJ-S2 outperforming CJ-S1. The 150IBU/Glc-S2 reactors achieved degradation efficiency and COD removal of $99.22 \pm 0.08\%$ and 79.16 ± 0.12 , respectively, while 150IBU/Glc-S1 reactors reached $80.84 \pm 0.01\%$ and 95.22 ± 0.03 , respectively. In the absence of Glc degradation performance decreased markedly. The 150IBU-S2 reactors achieved a degradation efficiency of $75.58 \pm 0.10\%$, whereas the 150IBU-S1 reactors reached only $46.69 \pm 0.07\%$. COD analysis confirmed these observations, with removal efficiencies of 56.13 ± 0.2 and $54.71 \pm 0.1\%$ for 150IBU-S1 and 150IBU-S2, respectively (Table 3). In contrast, biological controls revealed limited COD removal, with only 31.37 ± 0.3 and $13.43 \pm 0.3\%$ for 150IBU/Glc-S1 and 150IBU-S1 reactors, respectively. Overall, these results demonstrate significant IBU degradation under bioelectrochemical conditions, confirming the effectiveness of BES for the treatment of SPWW contaminated with IBU. Abiotic and open-circuit controls were analyzed by LC-MS to assess the degradation of IBU. No signs of transformation or elimination of IBU were observed under these conditions.

3.6. Bacterial Community Analysis

Figures 5–7 display the composition, relative abundance and diversity of bacterial communities in the sediments, biofilms, and electrolytes. At phylum level (Figure 5), both sediments were dominated by Pseudomonadota and Bacillota representing 89.4 and 8.3% for CJ-S1 and 92.8 and 3.7% for CJ-S2, respectively. In addition to these dominant phyla, CJ-S2 also contained low-abundance taxa such as Actinomycetota (2.31%). Biofilms and electrolytes were mainly colonized by Pseudomonadota (63–99%), while Bacillota were present at variable levels, reaching 34.84% in E60IBU/Glc-S2 and 3.19% in B150IBU-S2. Other phyla were detected in minor proportions (<2%) across all samples.

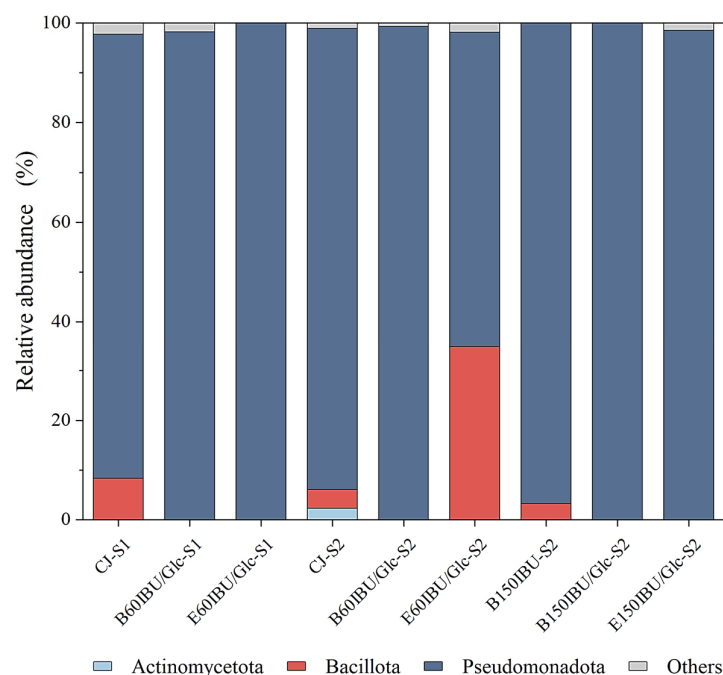


Figure 5. Bacterial distribution of biofilms (B60IBU/Glc-S1, B60IBU/Glc-S2, B150IBU/Glc-S2, and B150IBU-S2) and electrolytes (E60IBU/Glc-S1, E60IBU/Glc-S2, and E150IBU/Glc-S2); at phylum level, CJ-S1 and CJ-S2 are used as controls.

At the genus level (Figure 6B), the saline sediment CJ-S1 was predominantly composed of *Halomonas* (74.6%), *Idiomarina* (8.06%), *Halothiobacillus* (3.24%), *Bacillus* (2.81%) and *Halanaerobium* (2.11%). In contrast, CJ-S2 was strongly dominated by *Burkholderia* (90.29%). Biofilms and electrolytes were largely colonized by *Chromohalobacter*, with relative abundances ranging from 76.29 to 99.88% and from 63.61 to 92.21%, respectively. Other bacterial genera such as *Halomonas* (5.79–51%), *Bacillus* (2.81–14%), *Halanaerobium* (2.11–6.29%), *Rhodovibrio* (6.29%) and *Halothiobacillus* (3.24%) were also detected. Among all samples, E60IBU/Glc-S2 displayed the highest bacterial diversity, hosting a consortium dominated by *Halomonas* (51.31%), followed by *Bacillus* (14.47%), *Halanaerobium* (6.29%), *Tindallia* (5.82%) and *Rhodovibrio* (3.79%). This sample exhibited a notably reduced abundance of *Chromohalobacter*, suggesting a shift toward more functionally diverse taxa. When using CJ-S2 as inoculum, *Burkholderia* abundance decreased in all experiments, giving rise to the enrichment of genera associated with IBU degradation, particularly *Halomonas*, *Bacillus*, *Halanaerobium*, and *Rhodovibrio*. This enrichment was most pronounced under low IBU concentration (60 ppm) combined with Glc (20 ppm), which also yielded the highest current density ($38.02 \pm 0.15 \text{ mA m}^{-2}$).

An Upset plot (Figure 6A) was used to compare the genera present in all BES reactors. The *Chromohalobacter* was present in all BES samples, including biofilms and electrolytes, except in the electrolyte of the BES operated with 60 ppm of IBU and 20 ppm Glc using CJ-S2. *Halomonas* was identified in B60IBU/Glc-S1, E60IBU/Glc-S2, B60IBU/Glc-S2 and B150IBU-S2, while *Tindallia*, *Halanaerobium* and *Rhodovibrio* were found only in E60IBU/Glc-S2.

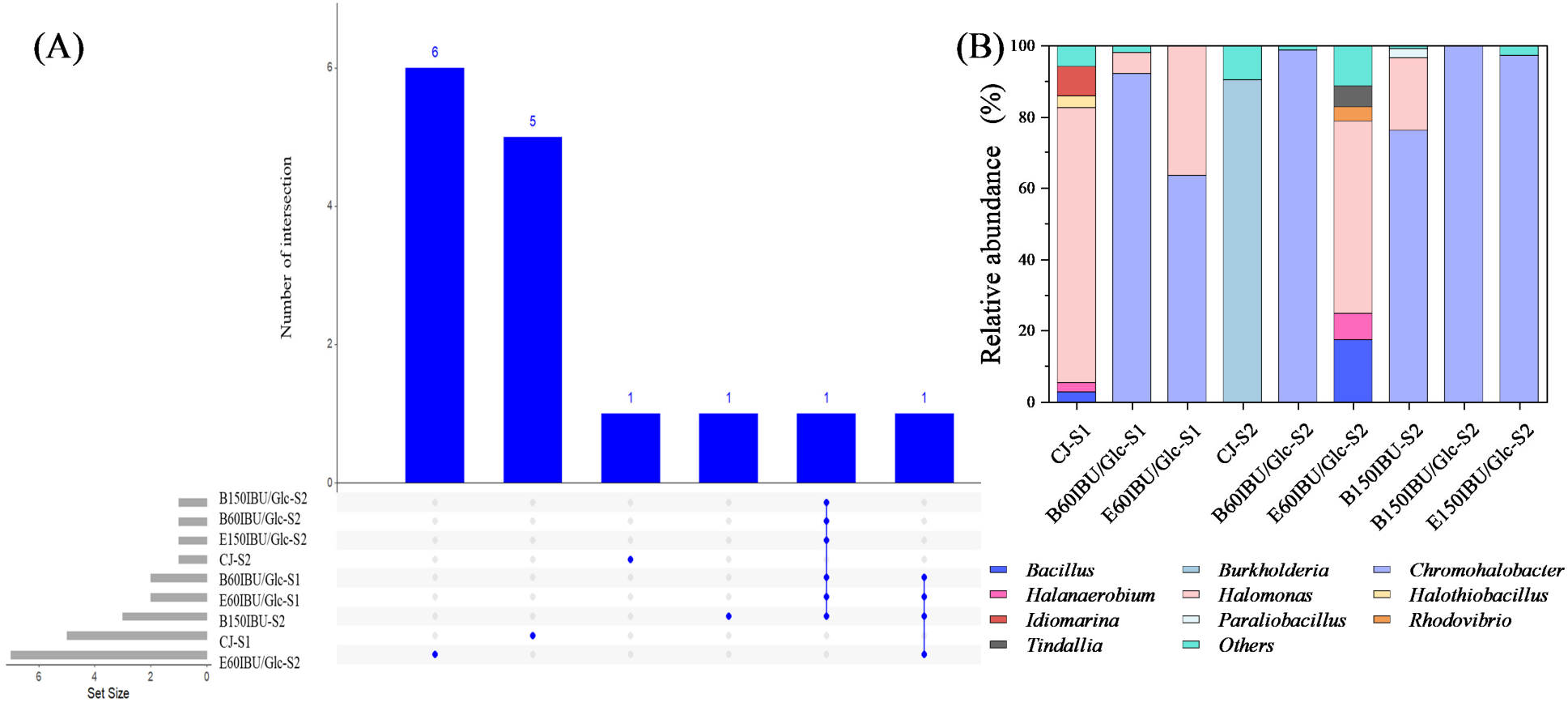


Figure 6. (A) Upset plot illustrates the number of shared genera in diverse conditions and (B) relative abundance of the main bacterial genus.

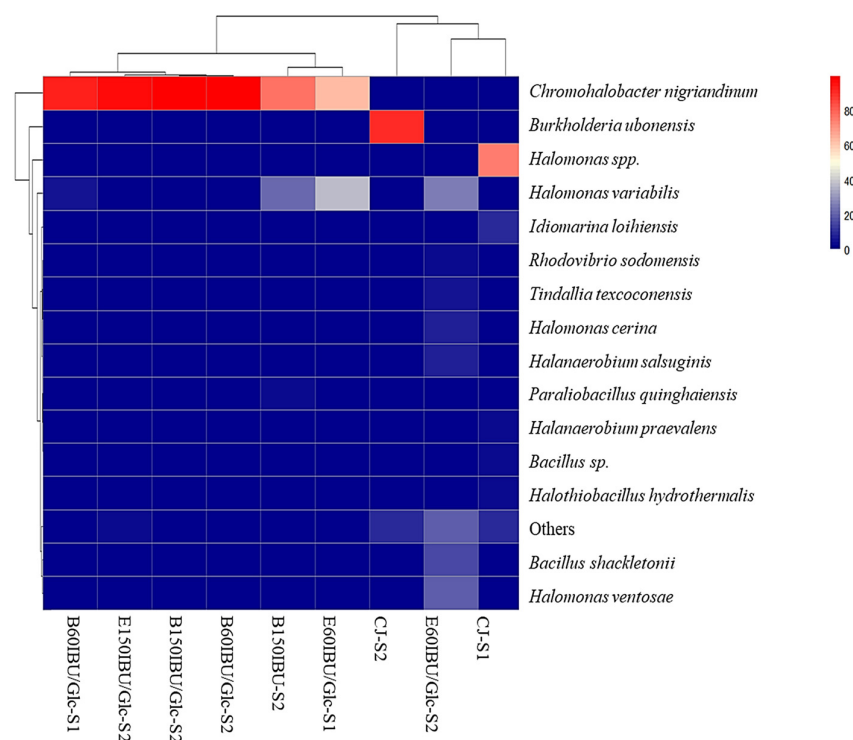


Figure 7. Heat map of distribution and phylogenetic connections of different species under distinct experimental conditions.

A heat map analysis (Figure 7) highlights the profiles of 16 species under different experimental conditions, showing phylogenetic relationships among species and distinct experimental conditions. The initial communities differed markedly between sediments: CJ-S1 was dominated by *Halomonas* spp. (60%), while CJ-S2 contained mainly *Burkholderia ubonensis* (80%). As shown in Figure 7, *Chromohalobacter nigriandinum* dominated most reactors (>80% relative abundance), except in E60IBU/Glc-S2 sample, which was enriched with *Halomonas variabilis* (40%), *Halomonas ventosae* (40%) and *Bacillus shackletonii* (20%). Samples B60IBU/Glc-S2, E150IBU/Glc-S2, B150IBU/Glc-S2 and B60IBU/Glc-S1 shared similar profiles dominated by *Chromohalobacter nigriandinum*. While samples B150IBU-S2, E60IBU/Glc-S1 and E60IBU/Glc-S2 showed some similarity with the enrichment of *Halomonas variabilis* (30–40%). Other species were detected with low abundance under all experimental conditions including *Halomonas cerina*, *Bacillus* sp., *Idiomarina loihiensis*, *Halanaerobium praevalens*, *Halanaerobium salsuginis*, *Tindallia texcoconensis*, *Paraliobacillus quinghaiensis*, *Halothiobacillus hydrothermalis* and *Rhodovibrio sodomensis*. Cluster analysis indicated that *Chromohalobacter nigriandinum* and *Burkholderia ubonensis* were very closely related in terms of abundance patterns, while *Idiomarina loihiensis*, *Halanaerobium praevalens*, *H. salsuginis* and *Paraliobacillus quinghaiensis* formed a distinct low-abundance cluster. As shown in Figure 7, the presence of Glc induced the strong selection of the bacterial community. For sample 150IBU-S2, in the absence of Glc, the bacterial community remained relatively diverse, with *Chromohalobacter nigriandinum* as the predominant species (76%), followed by *Halomonas variabilis* (20%). In contrast, the addition of Glc strongly induced the almost total dominance of *Chromohalobacter nigriandinum* in the biofilm (99.8%).

During the study of IBU degradation, we also focused on three crucial parameters: the effect of different concentrations of IBU (60 and 150 ppm), the effect of Glc as co-substrate and the distribution of the different halophilic consortia from CJ-S1 or CJ-S2. These parameters were analyzed (Figure 8A–D). Using CJ-S2 as inoculum, reactors with 60 ppm IBU and Glc exhibited the highest current density ($38.02 \pm 0.15 \text{ mA m}^{-2}$) and relatively high

community diversity (Shannon index = 2.57 in the electrolyte). Increasing IBU concentration to 150 ppm was associated with a decrease in Shannon diversity (Shannon index = 0.2 for the electrolyte and 0.11 for the biofilm), indicating a more selective enrichment of specific taxa under higher IBU stress, while the overall current density was only slightly reduced ($33.13 \pm 0.11 \text{ mA m}^{-2}$).

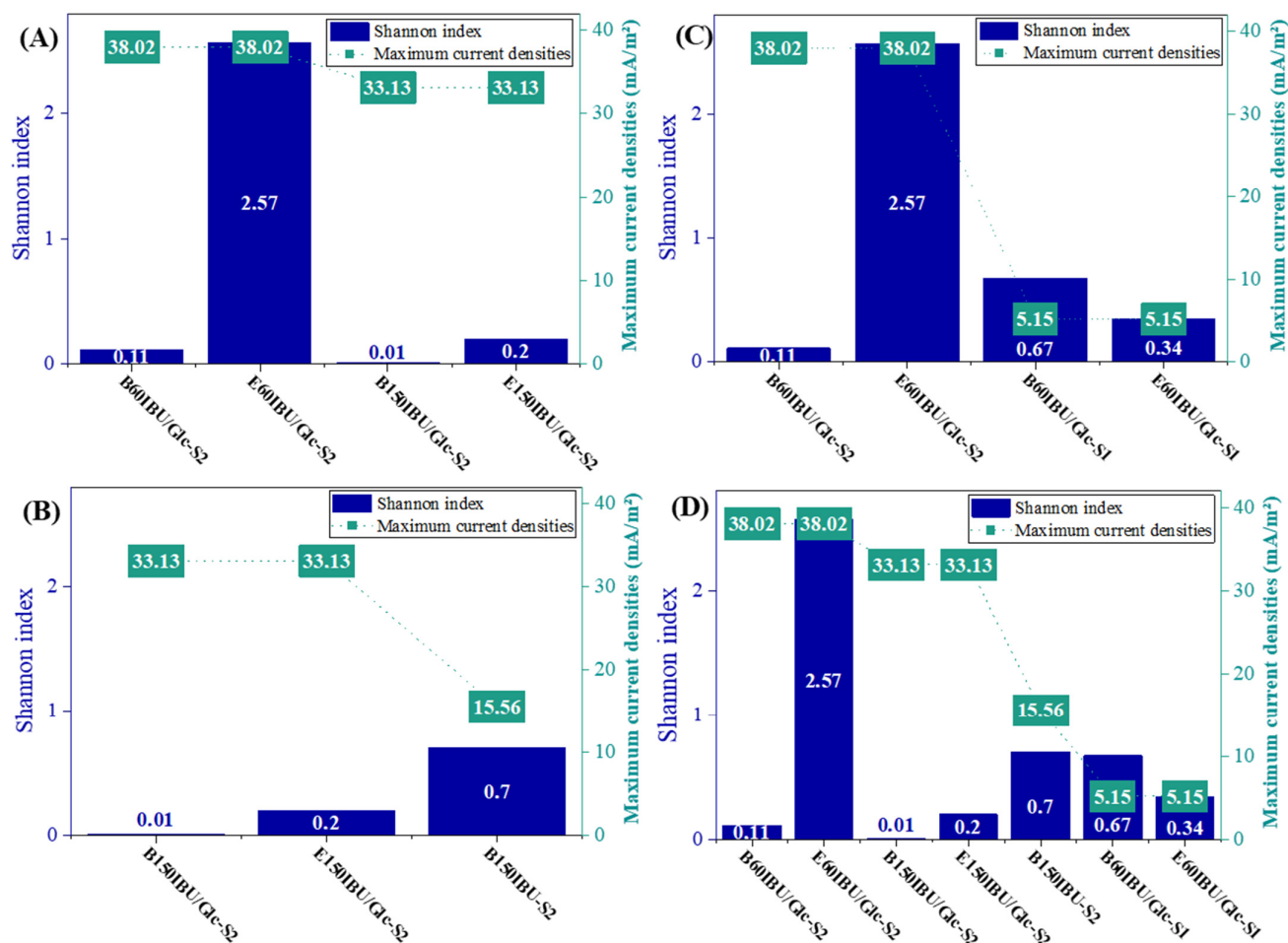


Figure 8. Study of different conditions: (A) variation in IBU concentration, (B) with or without co-substrat, (C) difference between CJ-S1 and CJ-S2 bacterial communities and (D) evaluation of IBU degradation under variable conditions.

Regarding the role of the co-substrate (Figure 8B), reactors without Glc recorded a current density of $15.56 \pm 0.06 \text{ mA m}^{-2}$ and a Shannon index of 0.7 (biofilm), whereas the addition of Glc doubled the current output ($33.13 \pm 0.11 \text{ mA m}^{-2}$) but resulted in lower diversity (Shannon index = 0.2 and 0.01 for electrolyte and biofilm, respectively). To assess the influence of the inoculum source, experiments conducted with CJ-S1 and CJ-S2 (both with 60 ppm IBU and 20 ppm Glc) revealed clear differences. Using CJ-S1, the current density reached only $5.15 \pm 0.03 \text{ mA m}^{-2}$ with Shannon indices of 0.34 (electrolyte) and 0.67 (biofilm). In contrast, CJ-S2 produced the highest current density with higher bacterial diversity (Shannon index values of 2.57 and 0.11 for electrolyte and biofilm, respectively). Overall, three main factors were particularly associated with variations in BES performance in this study: the IBU concentration, the presence or absence of Glc as co-substrate, and the source of the halophilic inoculum.

4. Discussion

4.1. Bioanode Performance and Influence of Substrate Concentration

Commercial IBU was used in this study to better reflect the actual state of the molecule as found in the environment and in industrial effluents. The IBU concentrations (60 and 150 ppm) used in this study were chosen to approximate the actual concentrations observed in pharmaceutical industry effluents discharged after the manufacturing process. In fact, wastewater representative of the pharmaceutical industry contains significantly high concentrations of IBU, reaching up to 400 ppm as reported for industrial effluents in Iran [13].

The performance of bioanodes in BES strongly depended on the used substrate, its concentration, and the inoculum source. Two different sediments were tested, and in all experiments, sediment was present; thus, differences in current densities could be attributed to the inoculum and the IBU concentrations. The control experiment carried out using only sediment CJ-S2 generated a higher current density ($93.9 \pm 0.02 \text{ mA m}^{-2}$), approximately three times higher than the maximum current density recorded in the presence of IBU, suggesting an inhibitory effect of IBU on bioanode activity or competition with the natural organic matter contained in the Chott Djerid sediment which provides biodegradable substrates for microorganisms and promotes efficient electron transfer. L. Joiner et al. [36] demonstrated that the total organic carbon (TOC) concentration in sediment is positively correlated with the energy performance of a benthic BES. A high TOC content implies a higher current production. The type of sediment inoculum strongly influences the current generation. In contrast, a clear inhibitory effect of IBU on the current generation was observed in this study, suggesting a reduction in bacterial metabolic activity. Similar inhibitory effects have been described by Zhu et al. [37], who found that low IBU concentration (1 ppm) significantly decreased bacterial diversity, with the Shannon index dropping from 3.42 to 2.69. In our study, the halophilic inoculum CJ-S2 achieved a maximum current density of $38.02 \pm 0.15 \text{ mA m}^{-2}$ with 60 ppm IBU and 20 ppm Glc, whereas CJ-S1 reached only $5.15 \pm 0.03 \text{ mA m}^{-2}$ under the same conditions. This difference in current production could be explained by the higher salinity and ionic content of CJ-S2, which enhances ion mobility and supports extracellular electron transfer (EET).

Although the cyclic voltammograms showed differences in redox activity between the reactors, these variations were not strictly proportional to the current densities obtained. For example, the voltammogram (A) in Figure S1 exhibited very similar initial and final cyclic voltammograms, despite generating the highest current density of $38.02 \pm 0.55 \text{ mA m}^{-2}$ and achieving an IBU degradation of $92.90 \pm 0.05\%$. This observation suggests that the evolution of the voltametric response is not necessarily directly correlated with the bioelectrocatalytic performance of the system [38]. This aligns with previous studies indicating that CV primarily characterizes extracellular electron transfer mechanisms and the electrochemical properties of electroactive biofilms, whereas CA is a more appropriate technique for evaluating the overall bioelectrocatalytic performance through current density measurements [38,39].

Moreover, the higher electrochemical activity observed with CJ-S2 suggests that it harbors a greater abundance of electrogenic and resilient bacteria compared to CJ-S1, emphasizing the importance of inoculum origin in BES performance. This indicates that electroactive bacterial community composition is a critical factor in current production and substrate degradation, consistent with prior studies [31,40]. Increasing IBU concentration to 150 ppm led to a significant drop in current density for CJ-S1, decreasing from $20.59 \pm 0.08 \text{ mA m}^{-2}$ with 60 ppm IBU to $8.45 \pm 0.06 \text{ mA m}^{-2}$ at 150 ppm IBU. These findings align with previous studies for other recalcitrant compounds. Saadaoui et al. [29] demonstrated a dramatic decrease in current density when dye concentration increased from

300 to 1000 ppm, and dropped from 12 to 0.19 A m^{-2} . Similarly, for Thiabendazole (TBZ) removal, Saidi et al. [27] reported a decrease in current density from 3.66 to 0.6 mA m^{-2} when TBZ concentrations increased from 50 to 150 ppm. Likewise, in petroleum hydrocarbon degradation, Venkidusamy et al. [41] observed a drop in current production from 180.21 to 114.54 mA m^{-2} when substrate concentration increased from 800 to 8000 ppm.

Previous studies have shown that IBU can influence bacterial physiology. According to Dai et al. [42], exposure of *Pseudomonas aeruginosa* to different concentrations of IBU (0, 50, 75, and $100 \mu\text{g mL}^{-1}$) resulted in a reduction in bacterial load proportional to the concentration of IBU. Furthermore, a significant reduction in the expression of genes associated with quorum sensing was reported, indicating a direct effect on the intercellular communication pathways essential for biofilm formation. Other studies have shown that treatment with IBU induced a metabolic reduction in *Staphylococcus aureus* of up to 80%, as well as a complete loss of the ability of adherent cells and biofilm growth [43]. Furthermore, exposure to IBU caused significant changes in intracellular K^+ release, suggesting destabilization and rupture of the cytoplasmic membrane. Moreover, ibuprofen isonobutanolammonium, at sub-inhibitory concentrations, altered the surface hydrophobicity of *E. coli* and inhibited fimbriae production, leading to a significant reduction in bacterial adhesion to uropathogenic cells.

A high recalcitrant substrate concentration could have a negative effect on current production which may be explained by the toxicity of the pollutants. It is essential to take into account both type and substrate concentration to improve BES performance in terms of degradation activities and the current production of persistent pollutants

Interestingly, unlike CJ-S1, increasing the IBU concentration to 150 ppm did not cause a substantial loss in current production in BES inoculated with CJ-S2. The system achieved $33.13 \pm 0.11 \text{ mA m}^{-2}$ at 150 ppm IBU with 20 ppm Glc, only slightly lower than the $38.02 \pm 0.15 \text{ mA m}^{-2}$. Based on these results, current density production in BES using THSCJ depends not only on the nature and concentration of the substrate, but also the bacterial community composition present in the sediment. Even sediments collected from the same site can exhibit different electrochemical performance if sampled at different times, due to temporal variations in community structure.

The addition of an easily metabolizable co-substrate such as Glc enhanced both current production and bacterial metabolism. In fact, Glc, as a standard co-substrate, serves as an additional electron donor, promotes the enrichment of electrochemically active bacteria, and accelerates pollutants transformation by enhancing metabolic pathways [44]. In enzyme-based biofuel cells, glucose oxidase and glucose dehydrogenase catalyze the oxidation of Glc to gluconolactone, producing electrons that move through an external circuit to access the cathode, enhancing current generation [45]. Sun et al. [46] reported an accelerated decolorization when Glc was used as co-substrate while degrading active brilliant red X-3B azo dye. More recently, Saidi et al. [27] examined the impact of TBZ and Glc on current production and removal efficiency. They recorded that the addition of Glc as co-substrate, even at low concentrations (30 ppm), boosts bacterial metabolism by providing an additional electron donor and enhanced the performance of bioelectrochemical process, achieving a maximum current density of 0.6 mA m^{-2} and TBZ removal efficiency of 99.23% [27]. As shown in Table 3, the coulombic efficiencies recorded in the bioelectrochemical experiments range from 6.54 ± 0.02 to 27.59 ± 0.14 . These results could be explained by limited efficient electron transfer in the BES or by the diversion of the electrons produced to other biochemical processes, which the electron-accepting bacteria could use for IBU reduction. Microorganisms can use Glc, as an easily assimilable substrate, for different metabolic pathways, such as anaerobic respiration, fermentation or biomass synthesis, thereby diverting a significant fraction of the electrons produced

from transfer to the electrode. Similar observations have been reported by Saidi et al. [27] showing that a low coulombic charge of 0.88 to 5.59 may be due to insufficient electron transfer in the BES or due to the diversion of electrons produced to other biochemical processes that electrogenic bacteria could use for TBZ reduction. These authors showed that bioelectrostimulation promotes synergistic interactions between electrogenic and non-electrogenic populations, allowing for the increased degradation of contaminants even when electron recovery in the form of current remains limited. These results confirm that coulombic efficiency primarily reflects the flow of electrons within the system rather than the specific degradation efficiency of IBU. In addition to substrate characteristics, sediment freshness and composition were decisive. Although CJ-S1 and CJ-S2 originated from the same hypersaline site, their physiochemical and bacterial properties differed clearly due to different sampling dates. CJ-S2 used immediately after collection had higher conductivity and higher concentrations of major ions (chloride, magnesium, potassium and iron) than CJ-S1. These properties contributed to the superior electrochemical activity observed with CJ-S2.

Control experiments performed under abiotic and in an open circuit showed no degradation of IBU, confirming that its transformation requires the participation of active microorganisms and a polarized electrode. In BESs, bioelectrostimulation combines electrode polarization and the addition of a co-substrate source that promotes bacterial metabolism and biofilm development, resulting in the effective degradation of IBU. These results highlight the crucial role of bioelectrostimulation in the elimination of persistent pharmaceuticals, particularly IBU. Recent studies have also shown that optimizing electrochemical interactions at the microbe–electrode interface and biofilm robustness are key factors in stimulating bacterial activity and electron transfer, confirming the effectiveness of bioelectrochemical approaches [47].

Microscopic analysis revealed that biofilm structure did not correlate directly with electrochemical performance. Although the biofilms formed in the 60IBU-S1 BES and the 150IBU/Glc-S1 BES (Figure 2A-60IBU-S1; Figure 2B-150IBU/Glc-S1) exhibited denser and more uniform colonization, they generated relatively low current densities. In contrast, the highest current was produced by biofilm formed in the 60IBU/Glc-S2 BES (Figure 2C-60IBU/Glc-S2) despite its moderate colonization level. This apparent contradiction indicates that current production is more related to the metabolic activity and electrochemical capacity of the bacterial community than to the physical density of the biofilm covering the working electrode. Under the 60IBU/Glc-S2 BES condition, the enhanced current associated with the formed biofilm suggests the predominance of highly electroactive species even at low abundance, whereas the more extensive colonization observed in biofilms formed in the 60IBU-S1 BES and the 150IBU/Glc-S1 BES likely reflects a higher proportion of non-electrogenic microorganisms. These results are consistent with previous studies that have shown that the physical structure of electroactive biofilms from hypersaline microbiome, whether they cover fibers or are dispersed between them, is not always predictive of the current produced [48]. Such morphology is typical of electroactive biofilms formed by inocula from hypersaline environments, where efficient electron transfer can occur even with limited biomass due to the dominance of highly electroactive bacterial communities. Overall, microscopy observations, combined with electrochemical data (Figure 1) and community analyses, suggest that biofilm composition and functional electroactivity, rather than biomass amount, govern current output in these designed hypersaline bioanodes.

4.2. Assessment of Ibuprofen Degradation

The present study aimed to evaluate biofilm development and determine the effectiveness of halophilic bacterial bioanodes in BES, compared to conventional biological

degradation, for the remediation of SPWW contaminated with IBU at concentrations of 60 and 150 ppm.

In the absence of Glc, the BES inoculated with CJ-S1 achieved almost complete IBU removal, reaching $99.98 \pm 0.02\%$ at 60 ppm. However, when the initial concentration increased to 150 ppm, a significant decline in BES performance was observed, with degradation dropping to $46.69 \pm 0.07\%$ (Table 3). This decrease suggests that higher IBU concentrations exert inhibitory effects on the bacterial community, limiting its metabolic capacity. COD removal followed the same trend, decreasing from $93.37 \pm 0.01\%$ at 60 ppm to $56.13 \pm 0.2\%$ at 150 ppm (Table 3). In contrast, the biological control showed substantially lower IBU degradation efficiency, with only $26.80 \pm 0.2\%$ COD removal at 60 ppm and $13.43 \pm 0.3\%$ at 150 ppm. This observation is consistent with the findings of Saidi et al. [31], who reported that weak electrostimulation applied to the same sediment CJS in BES increased TBZ degradation by approximately 40% compared to biological degradation alone. This pronounced difference confirms the essential role of electrostimulation in enhanced bacterial activity and enables the degradation of recalcitrant pharmaceutical pollutants such as IBU [30]. CJ-S2 performed markedly less efficiently in BES compared to CJ-S1, achieving moderate IBU degradation at 60 ppm ($51.79 \pm 0.12\%$) with COD removal of $56.79 \pm 0.3\%$. Moreover, at 150 ppm, IBU degradation increased to $75.58 \pm 0.10\%$, while COD removal remained relatively stable at $54.71 \pm 0.1\%$, indicating a somewhat unexpected response at higher pollutant concentration.

In the presence of Glc, IBU degradation was significantly enhanced under both concentrations. In BES inoculated with CJ-S1, IBU degradation remained extremely high at 60 ppm ($99.97 \pm 0.03\%$) and improved markedly at 150 ppm ($80.84 \pm 0.01\%$) compared to the Glc-free condition (Table 3). This enhancement clearly shows that Glc mitigates the inhibitory effects of high IBU concentrations by sustaining bacterial growth and enzymatic degradation pathways. Similarly, biological controls inoculated with CJ-S1 showed a positive Glc effect, where COD removal increased from 26.80 ± 0.2 to $38.07 \pm 0.03\%$ at 60 ppm and from 13.43 ± 0.3 to $31.37 \pm 0.3\%$ at 150 ppm. This demonstrates that Glc benefits the bacterial community even in the absence of electrostimulation, but the magnitude of the improvement is clearly limited without an applied potential. For CJ-S2, the positive effect of Glc was even more pronounced. At 60 ppm, degradation efficiency markedly increased, reaching $92.90 \pm 0.05\%$. At 150 ppm, BES achieved almost a complete IBU removal ($99.22 \pm 0.08\%$), surpassing CJ-S1 under the same conditions, suggesting that Glc addition allowed CJ-S2 to overcome its lower degradative capacity, potentially by activating otherwise degradative pathways or by enhancing electron availability for bacterial respiration.

As shown in Table 4, the obtained results corroborate previous studies emphasizing the role of Glc supplementation in BESs. Qin et al. [21] reported that Glc addition in a constructed wetland-microbial fuel cell (CW-MFC) achieved an IBU removal efficiency of 89.29%, while acetate supplementation as a co-substrate resulted in a lower removal efficiency of 62% [20]. This difference suggests that Glc more effectively enhances bacterial metabolic activity and facilitates extracellular electron transfer processes, thereby promoting IBU degradation.

Table 4. Comparative table of bioelectrochemical approaches for ibuprofen degradation (power density, Coulombic efficiency, removal efficiency, and COD removal rate).

[IBU] (ppm)	Type of Inoculum	Type of Co-Substrate	Type of BES	Anode Type	Max Current Density (mA m ^{−2})	Max Power Density (mW m ^{−2})	Coulombic Efficiency CE (%)	Removal Efficiency (%)	COD Removal Rate (%)	References
10	Activated sludge from the secondary sedimentation tank of the Tangshan WWTP in Nanjing	Glc	CW-MFC	Stainless-steel mesh incorporated within the activated carbon	ND *	75.50	ND	89.29	ND	[21]
1	anaerobic sludge	ND	Single-chamber microbial electrolysis cell	Graphite plates	ND	ND	ND	96.98	ND	[37]
50	Potting soil	ND	MFC	BCA	ND	4.26	ND	34	43.6	[49]
10	Anaerobic sludge	ND	Photo-MFC	Nanotubes/stainless steel	750	119	31	75.94	ND	[22]
5	<i>Eichhornia crassipes</i>	Acetate	CW-MFC	Granular activated carbon	ND	8.9	0.99	62	81	[20]
60	CJ-S1	Glc	Single-chamber microbial electrolysis cell	Carbon felt	5.15 ± 0.03	ND	6.54 ± 0.02	95.55 ± 0.03	95.55 ± 0.03	This study
	CJ-S2				38.02 ± 0.15	ND	27.59 ± 0.14	92.90 ± 0.05	93.46 ± 0.01	
	CJ-S1	Without co-substrate			20.59 ± 0.08	ND	19.86 ± 0.3	99.98 ± 0.02	93.37 ± 0.01	
	CJ-S2				8.59 ± 0.09	ND	9.06 ± 0.02	51.79 ± 0.12	56.79 ± 0.3	
150	CJ-S1	Glc			7.16 ± 0.05	ND	8.36 ± 0.1	80.84 ± 0.01	95.22 ± 0.03	
	CJ-S2				33.13 ± 0.11	ND	25.46 ± 0.16	99.22 ± 0.08	79.16 ± 0.12	
	CJ-S1	Without co-substrate			8.45 ± 0.06	ND	7.03 ± 0.01	46.69 ± 0.07	56.13 ± 0.2	
	CJ-S2				15.56 ± 0.06	ND	16.59 ± 0.3	75.58 ± 0.10	54.71 ± 0.1	

ND * Not determined.

In the present study, a superior IBU removal efficiency exceeding 99% was achieved, which is higher than the values commonly reported for CW-MFC [20,21] and Photo-MFC (75.94%) [22]. In addition to the remarkable removal performance, the BES achieved comparatively higher COD removal rates up to 95% compared to CW-MFC (81%) [21] and conventional MFC 43.6% [47], indicating enhanced organic matter degradation and improved overall treatment efficiency. Although performance variations may depend on operational conditions, reactor configuration, IBU concentration and inoculum type. The obtained results indicate that the robustness and potential applicability of the proposed system inoculated with halophilic microbiome.

FTIR analyses supported the chemical transformation of IBU after BES treatment. The disappearance of a characteristic band associated with carboxylic acid functional group confirms the cleavage of the C=O stretch and C-O bonds, which represents an essential first step in IBU breakdown. These observations are consistent with previous studies reporting FTIR band loss and shifts as evidence of IBU degradation [50]. Furthermore, the complete disappearance of multiple bands in the 520–1508 cm^{-1} region suggests that the aromatic ring of IBU has been altered and that functional groups, such as the isobutyl group or substituents on the benzene ring, have been transformed after BES treatment. The loss of these aromatic-associated peaks is significant, as the aromatic ring is one of the most recalcitrant structural elements of the IBU molecule. Overall, the FTIR results corroborate the LC-MS and COD findings, demonstrating the deep structural modifications consistent with advanced transformation and potential mineralization. The lack of clearly identifiable FTIR bands associated with residual IBU or potential transformations products does not necessarily confirm their complete removal. In addition, signals from possible degradation products can be obscured by overlapping bands originating from the complex wastewater, sediment matrix and other organic compounds. Furthermore, FTIR spectroscopy mainly provides information on variations in functional groups and does not allow the unambiguous identification of specific degradation intermediates. Furthermore, the BES operated with hypersaline sediments from Chott Djerid demonstrated high IBU removal efficiencies, outperforming conventional treatment technologies reported in the literature, while processes such as coagulation/flocculation [51], ultrafiltration [52], or chemically modified anodes in MFCs [49,53] generally achieve low to moderate IBU removal (<40%). In the present study, BES exhibits markedly higher removal performance, maintaining high efficiency even at elevated IBU concentrations, particularly in the presence of Glc. These performances are comparable to, or in some cases superior to, advanced oxidation processes such as UV/H₂O₂ and Fenton reaction [54], membrane processes including osmosis [55], and bacterial biodegradation [56], which operate at much lower IBU concentrations (<50 ppm) and often require high energy input, or chemical reagents. Previous studies also reported high IBU removal efficiencies using different bacterial strains, ranging from 60 to 100% at low concentrations (5–10 ppm) [57,58] and up to 100% at 200 ppm in bacterial electrochemical systems using activated sludge [59].

4.3. Influence of Bacterial Community Composition on BES Performance

Bacterial BESs are highly dependent on inoculum composition, as the presence, diversity, and metabolic capabilities of the bacterial community directly affect both electrochemical activity and the biodegradation performance of the target substrate [58]. Consequently, differences in bacterial composition can lead to substantial variation in current generation, biofilm formation, and pollutant removal.

In this study, both sediments used as inocula (CJ-S1 and CJ-S2), which exhibited markedly different initial bacterial profiles (Figures 5 and 6). Despite this, *Pseudomonadota* dominated all BESs treating SPWW contaminated with IBU (63–99%). This phylum has

previously been identified as dominant in BES inoculated with the same sediments during the degradation of azo dyes (86%) [30] and for fungicides [27]. This confirms the central role of Pseudomonadota in saline BES. At the genus level, CJ-S1 was initially dominated by *Halomonas* sp. (74.65%), whereas CJ-S2 was strongly dominated by *Burkholderia* (90.29%). After BES operation, however, distinct bacterial profiles emerged, reflecting the difference observed in IBU degradation and current density. As shown in Figure 7, a clear and consistent dominance of *Chromohalobacter nigrindinum* (63.61–99.88%) was observed in BES inoculated with either sediment at both 60 ppm and 150 ppm IBU, with or without Glc, specifically in BESs 60IBU/Glc-S1, 60IBU/Glc-S2, 150IBU-S2, and 150IBU/Glc-S2. In these reactors, IBU removal ranged from $75.8 \pm 0.10\%$ to $99.97 \pm 0.03\%$, indicating a strong association between the enrichment of *C. nigrindinum* and efficient IBU degradation. Notably, at highest IBU concentration (150 ppm), *C. nigrindinum* remained highly dominant (97.21%), demonstrating its remarkable tolerance to IBU toxicity and suggesting that this species can maintain high bioelectrochemical activity even under elevated pharmaceutical concentrations.

Several studies have reported that *Chromohalobacter* spp. tolerate and remove heavy metals [60], degrade endocrine disruptors as well as aliphatic and aromatic hydrocarbons including hexadecane in a saline environment [61], and mineralize hexachlorocyclohexane [62]. However, based on available literature up to late 2025, *Chromohalobacter nigrindinum* has never been identified as an IBU degrader nor as electroactive strain. The present work therefore represents the first report suggesting its dual role as an IBU-degrading and electrogenic bacterium, highlighting its potential for the bioremediation of saline pharmaceutical wastewater. In-depth investigation is being conducted to validate these results.

A notable exception was observed in the electrolyte of the BES operated at 60 ppm IBU with Glc using CJ-S2 as inoculum (Figure 7 E60IBU/Glc-S2). Here, *Chromohalobacter* was completely absent, and a varied consortium was enriched, including *Halomonas ventosae* (19.93%), *Bacillus shackletonii* (14.47%), *Halanaerobium salsuginis* (6.30%), *Halomonas cerina* (6.17%), and *Tindallia texcoconensis* (5.83%). A number of these genera are known degraders of toxic and persistent organic pollutants. For instance, *Bacillus* spp. is widely recognized for its ability to degrade aromatic compounds. Marchlewicz et al. [63] reported that a *Bacillus* strain could transform IBU primarily via hydroxylation of both the aromatic ring and aliphatic chain, producing hydroxy ibuprofen, although complete mineralization required a bacterial consortium, consistent with our detection of various complementary strains in this electrolyte. *Halomonas* spp. are also well-documented in BES inoculated with THSCJ, and known for degrading fungicides [31] and azo dyes [29], generating high power densities [64], and mineralizing the non-steroidal anti-inflammatory drug ketoprofen under aerobic conditions [65].

It was also observed that the *Burkholderia* strain was dominant in CJ-S2 sediments. However, by the end of the operation, its abundance decreased and *Chromohalobacter* became the dominant genus. This change is attributed to the halotolerant nature of *Burkholderia* [66], and in contrast to the superior halophilic adaptation mechanisms of *Chromohalobacter*. Vargas et al. [67] showed that *Chromohalobacter salexigens* is considered a moderately halophilic bacterium growing optimally between 0.5 and 2.5 M salt, with maximum growth at 1.5 M NaCl. In addition, *Chromohalobacter salexigens* is capable of rapidly utilizing a wide range of simple carbon compounds as sole sources of carbon and energy, conferring a strong metabolic flexibility under saline conditions. *Chromohalobacter salexigens* is characterized by the presence of at least five genes encoding betaine, ectoine, hydroxyectoine, and trehalose. These substances accumulate in high concentrations without

disrupting cellular metabolism, allowing the bacteria to survive and remain active in saline conditions.

A shift in the bacterial community developed at the biofilm level at 150 ppm IBU was observed depending on the presence or absence of Glc using CJ-S2 as a source of inoculum. Glc could also increase the bioavailability of hydrophobic substrates for microorganisms and enhance removal efficiency, enhancing the competitiveness of specific electroactive taxa and leading to a reduction in community diversity through competitive exclusion. Tyszkiewicz et al. [68] demonstrated that the choice of co-substrate, such as Glc, can have a significant influence on the composition and performance of the bacterial community in microbial fuel cell (MFC) systems. The presence of Glc significantly altered the structure of the anodic bacterial community, highlighting the impact of substrate availability on the diversity and dominance of specific taxa within the biofilm. Although the study focused on the degradation of benzene rather than ibuprofen, it showed that Glc addition led to the formation of distinct bacterial consortia with increased electrochemical activity compared to systems using other substrates, highlighting the importance of readily assimilable carbon sources in community dynamics and the performance of BESs [67].

Figure 8 further demonstrates that three key parameters strongly influence BES performance: pollutant concentration, inoculum origin, and co-substrate availability. Higher current production and bacterial richness were associated with lower IBU concentration (60 ppm), indicating the possible inhibition of electroactive bacteria at 150 ppm due to toxicity, which was observed with CJ-S1 inoculum. Although the addition of Glc greatly enhanced current density by promoting bacterial metabolism, it also decreased bacterial diversity, suggesting that only a few electroactive taxa predominated in these circumstances.

Overall, these findings confirm that BES performance depends not only on operational parameters such as pollutant concentration and co-substrate addition, but also critically on bacterial community composition. The enrichment of electroactive and specialized degradative bacteria, coupled with functional diversity and synergistic interactions, enables the efficient mineralization of IBU and stable bioelectrochemical activity. This underscores the central role of microbiome structure in determining BES efficiency for pharmaceutical wastewater treatment.

5. Conclusions

This study demonstrates the effectiveness of bioanodes developed from the hypersaline sediments CJ-S1 and CJ-S2 for the treatment of SPWW contaminated with IBU. The BES showed stable performance over 15 days, with the highest current density ($38.02 \pm 0.15 \text{ mA m}^{-2}$) obtained under optimal conditions combining 60 ppm of IBU, 20 ppm of Glc, and CJ-S2 as inoculum. IBU degradation was confirmed through complementary tools. FTIR analysis revealed significant spectral changes with peak disappearance, shifts, and the emergence of new bands, indicating molecular transformation and metabolite formation. LC-MS further validated this degradation, showing nearly complete removal (about 100%) under specific experimental conditions and confirming the breakdown of the parent molecule. Metataxonomic analysis of biofilms and electrolytes highlighted a strong enrichment of *Chromohalobacter nigrindinum* in most reactors, confirming its possible role in both electroactivity and biotransformation processes. Interestingly, the electrolyte of 60IBU/Glc-S2 displayed a distinct community dominated by *Halomonas* spp. and *Bacillus* sp. suggesting the emergence of an alternative functional consortium capable of supporting both IBU degradation and electron transfer. This work shows that BES treatment of SPWW is strongly shaped by the inoculum source, pollutant concentration, and the availability of carbon sources. The enrichment of *Chromohalobacter* spp. emerges as promising candidates

for the development of robust bioanodes suited for the remediation of saline wastewater containing persistent pharmaceuticals.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/chemengineering10080105/s1>, Figure S1. Cyclic voltammograms recorded on porous carbon felt electrodes immersed in reactors containing 60 or 150 ppm of IBU. In each panel, ICV denotes the initial cyclic voltammetry, whereas FCV denotes final cyclic voltammetry. (A) 60IBU/Glc-S2; (B) 60IBU-S2; (C) 60IBU/Glc-S1; (D) 60IBU-S1; (E) 150IBU/Glc-S1; (F) 150IBU-S1; (G) 150IBU/Glc-S2; (H) 150IBU-S2.

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Abbreviations

The following abbreviations are used in this manuscript:

SPWW	Synthetic pharmaceutical wastewaters
IBU	Ibuprofen
BES	Bioelectrochemical system
PPCPs	Pharmaceuticals and personal care products
NSAIDs	Non-steroidal anti-inflammatory drugs
Glc	Glucose
THSCJ	The Tunisian hypersaline sediments of Chott El Dejerid
WE	Working electrode
AE	Auxiliary electrode
SCE	Saturated calomel electrode
CA	Chronoamperometric
CV	The cyclic voltammetry
COD	Carbon oxygen demand
LC-MS	Liquid chromatography–mass spectrometry
FTIR	Fourier transform infrared
TOC	Total organic carbon
TBZ	Thiabendazole
MFC	Microbial fuel cell

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