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Article

Saline Sediments as a Suitable Source for Halophilic Inoculums to Degrade Azo Dyes in Synthetic and Real Textile Wastewaters by Microbial Electrochemical Systems

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Abstract: The treatment of textile wastewater (TWW) loaded with recalcitrant azo dyes in bioelectrochemical systems (BES) rather than in physicochemical processes is a low-cost and environmentally friendly process. The main objective of this study is to investigate the potential of different saline sediments collected from extreme Tunisian environments for the formation of bioanodes capable of simultaneous azo dyes degradation and electric current generation in synthetic (STWW) and real textile wastewaters (RTWW) characterized by a varied composition of azo dyes and a high salinity. The obtained bioanodes and anolytes were studied comparatively by electrochemical, microscopic, analytical, and molecular tools. Based on the UV–visible spectra analysis, the breakdown of the azo bond was confirmed. With RTWW, the BES achieved a chemical oxygen demand (COD) abatement rate of 85% with a current density of 2.5 A/m². Microbial community analysis indicated that a diverse community of bacteria was active for effluent treatment coupled with energy production. At the phylum level, the electrodes were primarily colonized by proteobacteria and firmicutes, which are the two phyla most involved in bioremediation. The analysis of the microbial community also showed the abundance of *Marinobacter hydrocarbonoclasticus* and *Marinobacter* sp. species characterized by their high metabolic capacity, tolerance to extremophilic conditions, and role in hydrocarbon degradation.

Keywords: microbial electrochemical systems; halophilic inoculums; textile wastewater; energy recovery; azo dyes degradation



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1. Introduction

Water pollution caused by the discharge of textile wastewater (TWW) is considered a serious environmental problem. TWW contains various dyes, salts at high ionic strength of about 5 to 6% NaCl and 5% of Na₂SO₄, and other chemicals, such as various acids, alkalis, sulfur, naphthol, surfactant-dispersing agents, formaldehyde-based dye fixing agents, and heavy metals [1,2]. World production of industrial synthetic dyes is estimated at more than 700,000 tons annually and about 10% left as residue are discharged into water bodies. Azo dyes constitute the largest class of synthetic dyes and represent 70 to 90% of the total synthetic dye market due to their color shades and low cost [3–6].

The discharge of TWW has major environmental consequences such as eutrophication, oxygen depletion, and loss of soil productivity. In addition, most chemical dyes have various drawbacks that seriously affect human health. Indeed, azo dyes enter the body through ingestion and cause cancer and DNA diseases [7,8]. Some of the chemicals in TWW including dyes are recalcitrant in nature because of their stability in front of the washing processes and therefore required treatment by appropriate and effective methods prior

to their release into the environment. Physical methods such as coagulation, flocculation, and filtration, chemical oxidation by oxidizing agents such as O_3 and H_2O_2 , advanced oxidation techniques such as photocatalytic oxidation and fenton, and biological methods using microorganisms such as fungi, algae, and bacteria [9–11] are the main treatment strategies explored to date. Nevertheless, physicochemical methods are not widely adopted because of their secondary pollution generation, large time demand, and high energy consumption [12,13]. Compared to conventional physical and chemical methods used for TWW treatment, microbial remediation is a suitable alternative that has the advantages of low operating costs (excluding aeration in aerobic bioprocesses) [14]. Unfortunately, microorganisms involved in the degradation of environmental contaminants generated by TWW have the shortcomings of a slow process [15].

The last few decades have seen the emergence of new bioelectrochemical systems (BES) aimed at reducing environmental impacts of hazardous and recalcitrant molecules such as dyes contained in TWW. Wu et al. [16] were the first to have successfully developed a BES for the continuous removal of Victoria blue R (VBR) dye applied in the textile industry and the simultaneous generation of electric current. The degradation rate of the dye was of 98.7% for artificial wastewater and 99.8% for real wastewater containing VBR. Naik et al. [17] also demonstrated that BES are a very effective method for removal of dyes, TDS (Total Dissolved Solid), TSS (Total suspended solids), COD (chemical oxygen demand), sulfates, and chlorides from sugar wastewater. Similarly, Hou et al. [18] reported the degradation of Congo red using glucose as co-substrate and found 97% removal efficiency for 300 ppm of dye. Sun et al. [19] generated electricity from the co-oxidation of simple substrates and azo dye in a single chamber microbial fuel cell (MFC). They found that glucose compared to acetate was the best co-substrate for ABRX3 decolorization. Recently, complete decolorization of the azo dye methyl orange using an electrochemical approach was recently reported in the study by Sarfo et al. [20], as well as an 83% reduction rate of total organic carbon using a simple Cu sheet as a working electrode.

Despite several exploratory or applied works that have been carried out on the decolorization of azo dyes by BES, no specific research has focused on the degradation of Tubantin blue (TB) and Bezaktiv Red (BR), which are plentiful in TWW due to their intensive use by textile industries. Both azo dyes with other organic and inorganic compounds in TWW constitute a peculiar reservoir of chemical energy that theoretically could be converted into electrical energy in BES. Already in this perspective, a previous study by Askri et al. [21] demonstrated the possibility of enriching powerful exo-electrogenic microorganisms on bioanodes capable of removing COD by $91 \pm 3\%$ and producing a current density of $12.5 \pm 0.2 \text{ A/m}^2$ from the hypersaline sediments of Chott El Djerid (SCD) and real TWW.

Subsequently, the novel objective of this study was to design microbial bioanodes capable of treating (i) synthetic textile wastewater (STWW) and (ii) real textile wastewater (RTWW) containing the two azo dyes TB and/or BR using an electromicrobial process involving electroactive halophilic bacteria already adapted in hypersaline sediments of extreme Tunisian environments. The contribution of electromicrobial bioremediation was systematically compared to a conventional microbial bioremediation control. The addition of an organic co-substrate that can serve as an electron donor and initiate microbial growth was investigated. The fate of azo dyes was tested at initial concentrations of 300 and 1000 ppm, individually and in combination.

After the enhanced degradation of azo dyes in both STWW and RTWW has been proven by electrochemical (Chronoamperometry (CA), Cyclic voltammetry (CV)) and spectroscopic (UV-VIS, FTIR) tools, the contributions of anodic bacteria enriched with SCD or SSM are studied by scanning electron microscopy (SEM) imaging of biofilm organization on carbon felt electrodes and by comparative metagenomic analyses of the different biofilms and inocula.

2. Materials and Methods

2.1. Collection of Dyes, Real Textile Wastewater (RTWW), and Hypersaline Sediments

Tubantin Blue BRR H.C (TB) and Bezaktiv Red S-Matrix 150 (CHT group in Tübingen, Germany) (BR), used as ingredients for STWW, were collected from the Global Washing textile industry Gitex group located in Korba, Nabeul, Tunisia [36°34'24.636'' N and 10°51'31.463'' E]. RTWW was collected from the same industry. RTWW is discharged just after the tanning process into a pond. The effluent was collected from this pond and then stored in drums at +4 °C until experiments were started.

The hypersaline sediments used as sources of halophilic inoculums were sampled from the sediment of Chott El Djerid (SCD) [33°59'965'' N and 08°25'332'' E] and the sediment of Sebkhath El Melah (SSM) [33°23'4.192'' N and 10°57'51.468'' E], both located in the south of Tunisia. All samples were conserved in closed plastic bags and bottles at +4 °C until experiments were started.

2.2. Bioelectrochemical System Setup Construction and Electrochemical Data Processing

All electrochemical experiments were performed in a 750 mL reactor containing 80% of STWW or RTWW and 20% of SCD or SSM. After a homogenization step by mechanical mixing, pure nitrogen gas was bubbled through the anolyte for 30 min to remove soluble oxygen.

A conventional 3-electrode device (working electrode, counter electrode, and reference electrode) was used with a MGP multichannel potentiostat (Biologic SAS, Seyssinet-Pariset, France) equipped with EC lab software. The working electrode made of porous carbon felt of 6 cm² projected surface area was connected to a titanium rod (1 mm diameter and 15 cm long) and polarized at −0.1 V/SCE, a platinum grid was used as the counter electrode, and a saturated calomel reference electrode (+0.24 V/SHE) was located between the counter and the working electrode [21,22].

STWW was composed of 80% of enrichment medium containing the following (g/L): 0.5 K₂HPO₄, 1.0 NH₄Cl₂, 2.0 MgCl₂, 0.1 CaCl₂, NaCl (165 or 15 g), 20% of SCD or SSM, and an azo dye at two different concentrations of 300 and 1000 ppm for STWW. Acetate and glucose at a concentration of 2000 ppm and 500 ppm were used as co-substrates to determine their effect on dye removal and current generation. For the RTWW experiments, reactors were inoculated with 80% of RTWW and 20% of SCD or SSM. The initial pH in all experiments was adjusted to 7.2 with 3M NaOH.

For the electrochemical analysis of forming bioanodes, chronoamperometry (CA) and cyclic voltammetry (CV) were used. For the CA, the working electrodes were polarized at −0.1 V/ECS. The CV was performed in situ after the CA was stopped between −0.6 and +0.3 V/SCE at a scan rate of 1 mV/S at the beginning and at the end of each experiment.

2.3. Monitoring of Decolorization, Quantification of COD, and Measurement of Azo Dye Biodegradation

Samples were collected from the anolyte every 48 h and were centrifuged at 12,000 rpm for 5 min. The decolorization rate was monitored by measuring the decrease in absorbance of the anolyte free of suspensions with a UV-VIS spectrophotometer (Jenway 7315, Antylia Scientific, Villepinte, France) at the wavelengths of 556 nm for TB and 520 nm for BR.

A sample of 1 mL was taken every 48 h from each anolyte, diluted at 1/6, and filtered through the syringe of the chloride elimination LCW925 kit (Hach, Loveland, CO, USA). The COD removal efficiency was determined using the LCK 514 kit (100–2000 mg/O₂) (Hach, Loveland, CO, USA).

Dye biodegradation was monitored by Fourier Transform Infrared spectroscopy FTIR (Perkin Elmer model). Samples from anolyte were taken at the beginning and at the end of the experiment. FTIR analyses were conducted in the mid IR region of 400–4000 cm^{−1}.

2.4. Microscopic Characterization of Biofilms

The biofilm morphologies on the surface of the bioanode were examined by SEM-FEG scanning electron microscope (SEM). Before SEM observation, bioanodes were treated as described by [23]. Bioanodes were fixed in phosphate buffer (400 mM, pH = 7.4) with 4% glutaraldehyde. They were rinsed in phosphate buffer containing saccharose (0.4 M) and dehydrated by immersion in increasing concentrations of acetone (50, 70, and 100%), then in acetone and hexamethyldisilane (HMDS) (50:50%), and in 100% HMDS. The last batch of HMDS was dried until complete evaporation.

2.5. Bacterial Community Analyses

Genomic DNA extractions were performed from different sediments using a NucleoSpin® kit and from different biofilms using DNeasyPowerBiofilm Kit (Qiagen, Hilden, Germany). The purity [absorbance ratio (A_{260}/A_{280})] and DNA concentration measurements ($\text{ng}/\mu\text{L}$) were checked by Nanodrop TM 2000 spectrophotometer (ThermoFisher Scientific, Waltham, USA) [21]. Then, Illumina Miseq 16S rRNA sequencing was performed in order to analyze the composition of the bacterial communities in sediment samples and in different biofilms. The 16S rRNA gene V4 variable region PCR primers 515/806 were used in single-step 30 cycles PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, Hilden, Germany). Sequencing was performed at MR DNA (<https://www.mrdnlab.com/www.mrdnlab.com>; accessed on 23 April 2023; Shallowater, TX, USA).

3. Results and Discussion

3.1. Decolorization of Tubantín Blue (TB)

Decolorization of TB and current generation in STWW were tested at two initial dye concentrations 300 (STWW1) and 1000 ppm (STWW2) using a concentration of 2000 ppm as a co-substrate and two different sources of halophilic inoculums—SCD and SSM. As shown in (Figure 1A(a)), halophilic inoculum from SCD was able to generate in short time a maximum current density of $12 \text{ A}/\text{m}^2$ under salinity of 165 g/L with a decolorization rate of 84% achieved in 12 days for STWW1 (Figure 1B(a)). However, when increasing the concentration of TB from 300 to 1000 ppm (STWW2), the decolorization decreased from 84 to 55%, suggesting that the decolorization rate in STWW1 was more important than in STWW2 (Figure 1B(b)). Consequently, increasing the initial concentration of the dye resulted in a weak decolorization, which may be caused by a greater accumulation of the dye.

These results indicate that decolorization performance and electrochemical yield are affected by high dye concentration. Sun et al. [19] also observed a decrease in decolorization rate starting from 800 ppm of azo dye. They attributed this decrease to a bacterial inhibition growth caused by dye toxicity. Recently, Qiu et al. [24] used a halotolerant microflora to treat a complex textile effluent composed of five azo dyes. This microflora was able to tolerate a salt concentration ranging from 0 to 80 g/L. In comparison with the above-mentioned study, it was found that despite the use of twice the salt concentration, the rate of decolorization was approximately similar, suggesting a high tolerance to the extreme condition. The authors confirmed the advantage of using the extreme halophilic consortia over the moderately halophilic microflora. The effect of the initial dye concentration was also investigated. Indeed, at concentrations maintained between 200–600 mg/L, the decolorization rates exceeded 70% (very close to the rates obtained with STWW 1 in our study). On the other hand, increasing this concentration resulted in a significant decrease in the discoloration rates caused mainly by bacterial toxicity.

For experiments using SSM as source of halophilic inoculum, 70% decolorization rate was obtained in 12 days and low maximal current density of $0.1 \text{ A}/\text{m}^2$ was recorded when the reactor was fed with STWW1 (Figure 1A(c),B(c)). These findings demonstrated that SSM inoculum may harbor more tenacious and electroactive community than SCD inoculum towards STWW1 experiments.

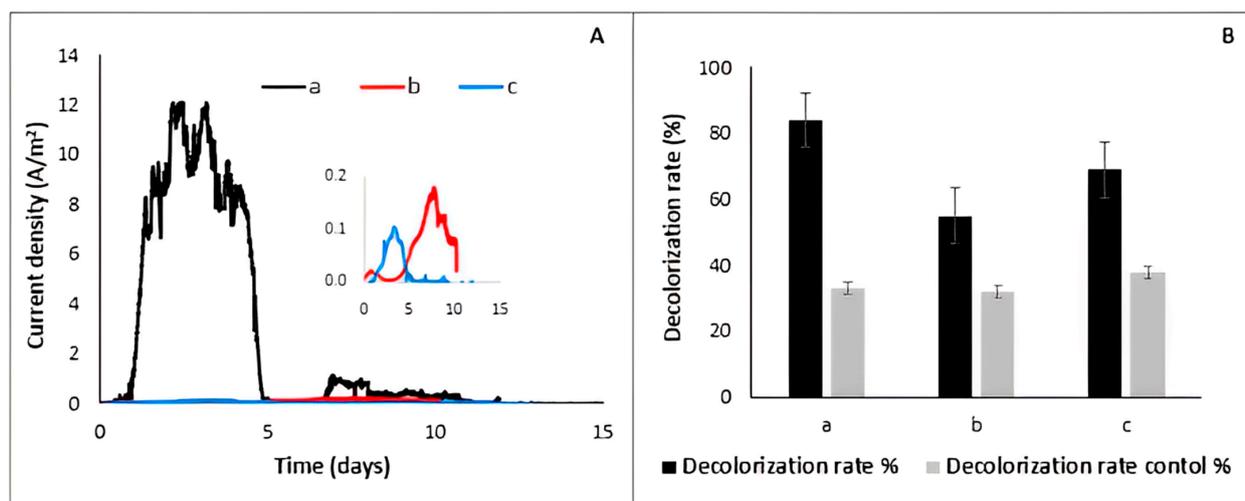


Figure 1. (A) Evolution of the anodic current recorded on the 6 cm² carbon felt working electrode polarized at -0.1 V/SCE; and (B) decolorization rate achieved by bioremediation or bioelectroremediation of STWW. (a) Reactor fed with 300 ppm TB,SCD as source of halophilic microorganisms; (b) Reactor fed with 1000 ppm TB, SCD as source of halophilic microorganisms; (c) Reactor fed with 300 ppm TB, SSM as source of halophilic microorganisms. All experiments have been performed at a salinity of 165 g/L.

Considering the visual aspects of the two sediments, the SCD appeared more heterogeneous than the SSM with a mixed local hydration, and three different colors (black, brown, and beige). On the other hand, the SSM appears more homogeneous with only one color, and without hydrated zones. Additionally, the sediment chemical analysis showed that the SCD naturally contained more concentrated mineral elements (Table 1). Indeed, the concentrations of calcium, magnesium, potassium, and iron expressed in mg/kg of dried materials in SCD were higher than those of SSM. The difference in azo dye decolorization and anodic electrical production performances of the two sediments could probably be related to their specific difference in mineral composition. In fact, particular mineral composition of SCD leads to more favorable environment for the development of azo dye-degrading microorganisms.

Table 1. Composition of mineral elements in the two SCD and SSM sediments.

Composition (g/kg DM)	Sediments	
	SCD	SSM
Calcium	196.3	130.0
Magnesium	9.5	6.3
Potassium	3.2	1.8
Sodium	35.2	54.8
Iron	4.5	2.1

DM: dried materials.

When glucose was used as co-substrate with a concentration of 500 ppm under salinity of 165g/L, SCD as inoculum, and 300 ppm of TB, the decolorization rate was 78% after 16 days and current density varied from 3 to 3.7 A/m². Experiments performed with acetate under the same conditions showed a very weak rate of decolorization (21%) and current production of 0.7 A/m² (Figure 2A,B). These results show that the decolorization rate when acetate is used as a co-substrate was about four times lower compared to its analogue (glucose); acetate may act as an inhibitor of TB decolorization. According to the literature, acetate appears to be the less efficient electron donor for the bioelectrochemical treatment of azo dyes. In fact, acetate seems to be a poor co-substrate with minimal decolorization rate. This can be attributed to the fact that acetate and other volatile fatty acids are normally

poor electron donors, whereas glucose is a more efficient electron donor for the reduction inazo dyes [18,25,26]. The negative effect of acetate on the anoxic decolorization could be attributed to a catabolic repression. As reported by Sandhya et al. [27], acetate probably inhibited cyclic AMP-dependent genes, including those coded for azoreductases.

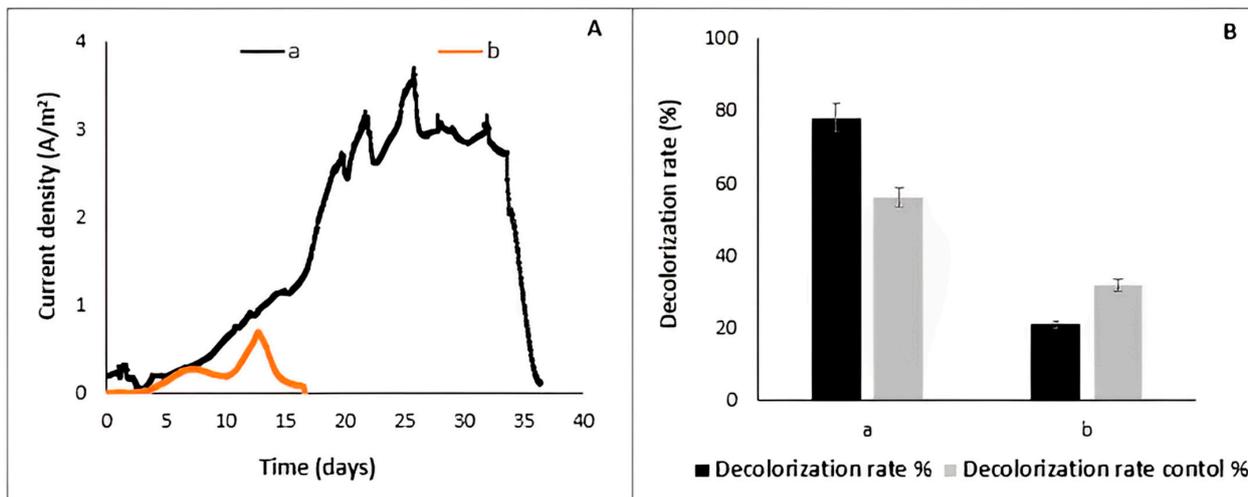


Figure 2. (A) Evolution of the anodic current recorded on the 6 cm² carbon felt working electrode polarized at -0.1 V/SCE; and (B) decolorization rate achieved by bioremediation or bioelectroremediation of STWW. (a) Reactor fed with 300 ppm TB, SCD as source of halophilic microorganisms, glucose as co-substrate; (b) Reactor fed with 300ppm TB, SCD as source of halophilic microorganisms, acetate as co-substrate.

In order to detect electroactive redox systems that may be related to the formation of a biofilm or microbial cells on the surface of the working electrode, cyclic voltammeteries (CVs) at the beginning and at the end of each experiment were established. In all cases, the initial CVs showed the absence of any electrochemical reaction on the conductive surface of the carbon felt electrode as shown by the voltammograms' trend in the potential range from -0.1 to 0.3 V/SCE (Figure 3). However, a reduction current was observed from -0.1 to -0.6 V/SCE, which probably corresponds to the electrochemical reduction in the soluble oxygen residue trapped into felt porosity, and not totally removed when initially purging with N₂ gas (Figure 3A). Further non-turnover CV cycles were performed at the end of the experiments. A high-capacitive current, and two redox systems at -0.1 and -0.2 V/SCE, were clearly identified, suggesting the establishment of a microbial electroactive biofilm on the surface of the carbon felt (Figure 3A,B).

3.2. Decolorization of Bezaktiv Red (BR)

Both sediments SCD and SSM were also tested as microbial inoculum in order to form electro-active microbial bioanode under two different concentrations of BR in synthetic wastewaters named STWW1 (300 ppm) and STWW2 (1000 ppm).

A decolorization rate of 55% was recorded in 5 days when STWW1 was treated by SCD as inoculum. During this period, no current density was recorded in front of a total absence of decolorization in the controlled reactor (Figure 4A(a)). Addition of glucose as a co-substrate allowed a significant acceleration in the decolorization of BR (99%) during day 7, with current production of 3.6 A/m² stable for 4 days (Figure 4A(a),B(a)). However, we obtained a lower decolorization rate (less than 55%) when used STWW2 and current production less than 0.1 A/m² was recorded (Figure 4A(d),B(d)). These findings indicated that increased dye concentration from 300 (STWW1) to 1000 ppm (STWW2) could decrease the performance of the BES. For experiment conducted with SSM as source of halophilic microorganisms, STWW1, and glucose as co-substrate, the decolorization rate was 70%, with a decrease in efficiency of about 30% compared to results obtained with SCD. In

these conditions current generation was very weak (less than $0.05\text{A}/\text{m}^2$), as shown in Figure 4A(b),B(b). Additionally, Figure 4A(c),B(c) summarizes the results of the experiment performed with STWW1 at a salinity of $15\text{ g}/\text{L}$ and using SCD as the inoculum source. A current generation of $1\text{A}/\text{m}^2$ was obtained with an almost complete decolorization (98%). These findings confirmed that BR complete decolorization may require the presence of an electron donor co-substrate.

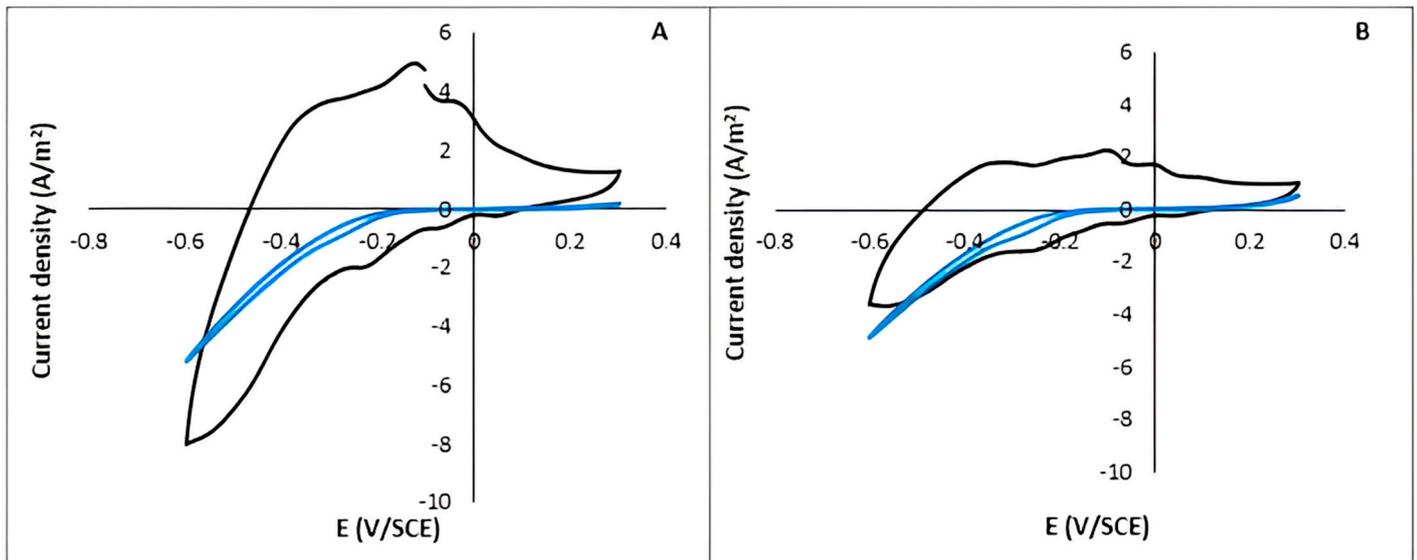


Figure 3. Cyclic voltammeteries performed on porous carbon felt electrode immersed in reactor contained 300 ppm of TB. (A) SCD as inoculum and glucose as co-substrate. (B) SSM as inoculum and glucose as co-substrate. CV_i (–), CV_f (–).

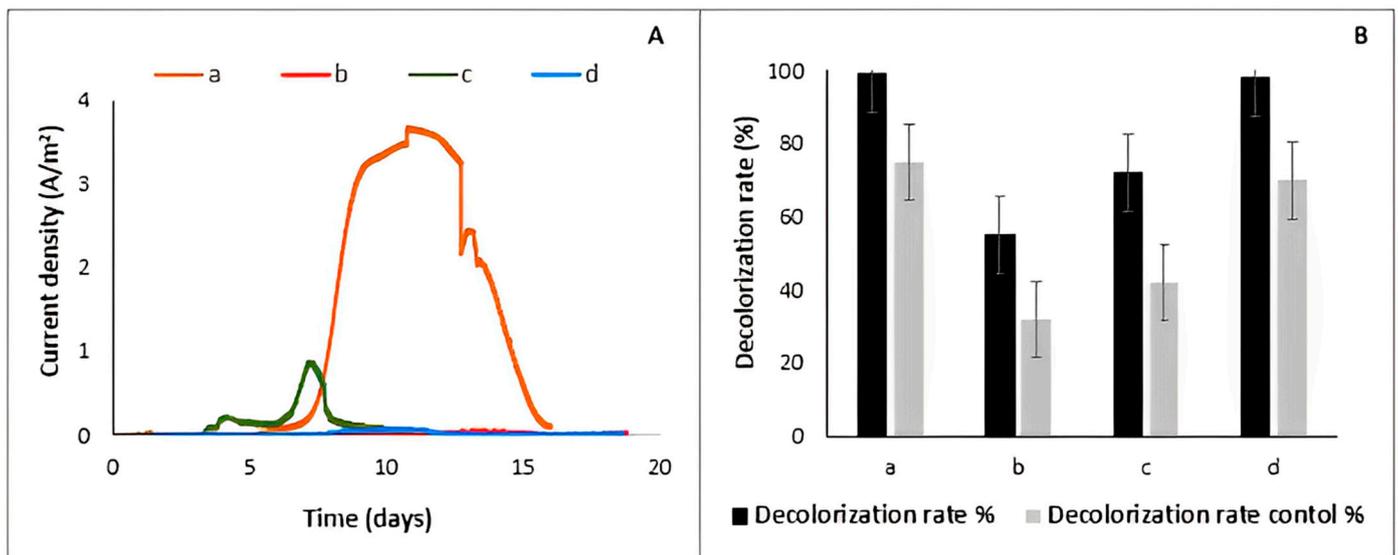


Figure 4. (A) Evolution of the anodic current recorded on the 6 cm^2 carbon felt working electrode polarized at $-0.1\text{ V}/\text{SCE}$; and (B) decolorization rate achieved by bioremediation or bioelectroremediation of STWW. (a) Reactor fed with 300 ppm BR, SCD as source of halophilic microorganisms; (b) Reactor fed with 1000 ppm BR, SCD as source of halophilic microorganisms; (c) Reactor fed with 300 ppm BR, SSM as source of halophilic microorganisms. All experiments have been performed at a salinity of $165\text{ g}/\text{L}$ substrate. (d) Reactor fed with 300 ppm BR, SCD as source of halophilic microorganisms at a salinity of $15\text{ g}/\text{L}$.

For TB and BR decolorization, we found that decolorization rate and current production were hindered as soon as the dye concentration was increased. In fact, the decolorization rate decreased from 84 to 69% and from 99 to 55% when concentrations of TB and BR were increased from 300 to 1000 ppm in synthetic wastewaters. Nevertheless, it is interesting to note that with STWW2, the amount of dye material lost during the bioelectrochemical treatment process was two times higher than that observed with STWW1 (99 vs. 55%). In addition, with STWW2, a dramatic inhibition of current generation was noticed. Based on the mechanisms of azo dye degradation involving competition between electron transfer to the electrode or to the N=N bond, more azo dye concentration theoretically leads to low anodic current production. Here, 1000 ppm concentration of BR generated less electron transfer to the anode and consequently decreased current density. Similarly, Thung et al. [28] reported that an increase in the initial concentration of orange Ao7 from 50 to 75 ppm lead to a decrease in decolorization rate from 80.6 to 75.0%. Miran et al. [29] also obtained decolorization rates of 91, 86, 81, 73, and 67% at initial dye concentrations of 100, 250, 500, 750, and 1000 ppm, respectively. Recently, Shahi et al. [6] also noticed that concentrations of reactive orange above 500 ppm are considered as inhibitive concentrations for decolorization and electricity generation.

3.3. Monitoring of Decolorization of TB and BR in RTWW: COD and FTIR Analyses

It should be mentioned that there are few attempts to treat RTWW with BES; most studies have focused on STWW [30]. In this study, we have evaluated the BES using STWW and then validated with RTWW containing naturally the same dyes used in STWW treatment experiments with a concentration that varies from 300 to 500 depending on the tanning process. The RTWW containing TB and BR among other constituents (0.7 mg/L anionic detergents, 9×10^3 mg/L chlorides, 777 mg/L sulfates, and 102 mg/L suspended matters) was fed into the BES with SCD or SSM inoculums. Attempts to form a bioanode were conducted by chronoamperometry at -0.1 V/SCE. Constant electrode polarization was maintained for 30 days and 15 days, respectively, when SCD and SSM were used as inoculum. A maximal current production of 2.0 and 0.8 A/m² were obtained, respectively (Figure 5(a)).

As shown in Figure 5C,D, initial CVs clearly show the absence of an electroactive biofilm; reduction currents were recorded from -0.2 V, which corresponds to the electrochemical reduction in some compounds present in the reaction medium to be treated; during this phase, the biofilm has not yet reached its maturity. Final CVs were performed at the end of each experiment; remarkable differences in the general appearance of the CVs were identified compared to their initial states, confirming the activity of biofilms; the oxidation plateau was maintained in the range of -0.2 to $+0.3$ V with maximum peaks of 4 and 2 A for SCD and SSM, respectively. The CVs results clearly demonstrated the formation of a biocatalyst biofilm on the conducting surface for a polarization period that exceeded 15 days.

It is noteworthy that TB and BR decolorization rates obtained in the case of RTWW treatment are comparable to those observed when using glucose as co-substrate with STWW experiments. Based on these data, it is apparently unnecessary to add an additional co-substrate to initiate the cometabolism reaction with RTWW. In fact, carbohydrates and other complex organic materials present in RTWW could be used by the microbial community to increase the electrochemical process. It is estimated that the concentration of carbohydrates in textile effluent could reach up to 22.000 ± 0.151 mg/L [31]. In fact, the water evacuated during the tanning process carries a significant chemical load that comes from the sizing products—mainly starches and their derivatives, carboxymethyl cellulose, and galactomannans. In addition, various additives are present in the dye formulations. Since these substances are not absorbed or fixed by the fibers, they are discharged into the wastewater with considerable COD load. Such additives are buffer systems composed of acetate, thickeners composed of carboxymethyl cellulose, and dispersants composed of ethylene oxide copolymers and detergents [32]. Based on these data, the treatment of real textile wastewaters in a BES may not require the addition of a co-substrate to enhance the

co-metabolism reaction. This could significantly reduce the treatment cost by avoiding the mobilization and addition of co-substrates.

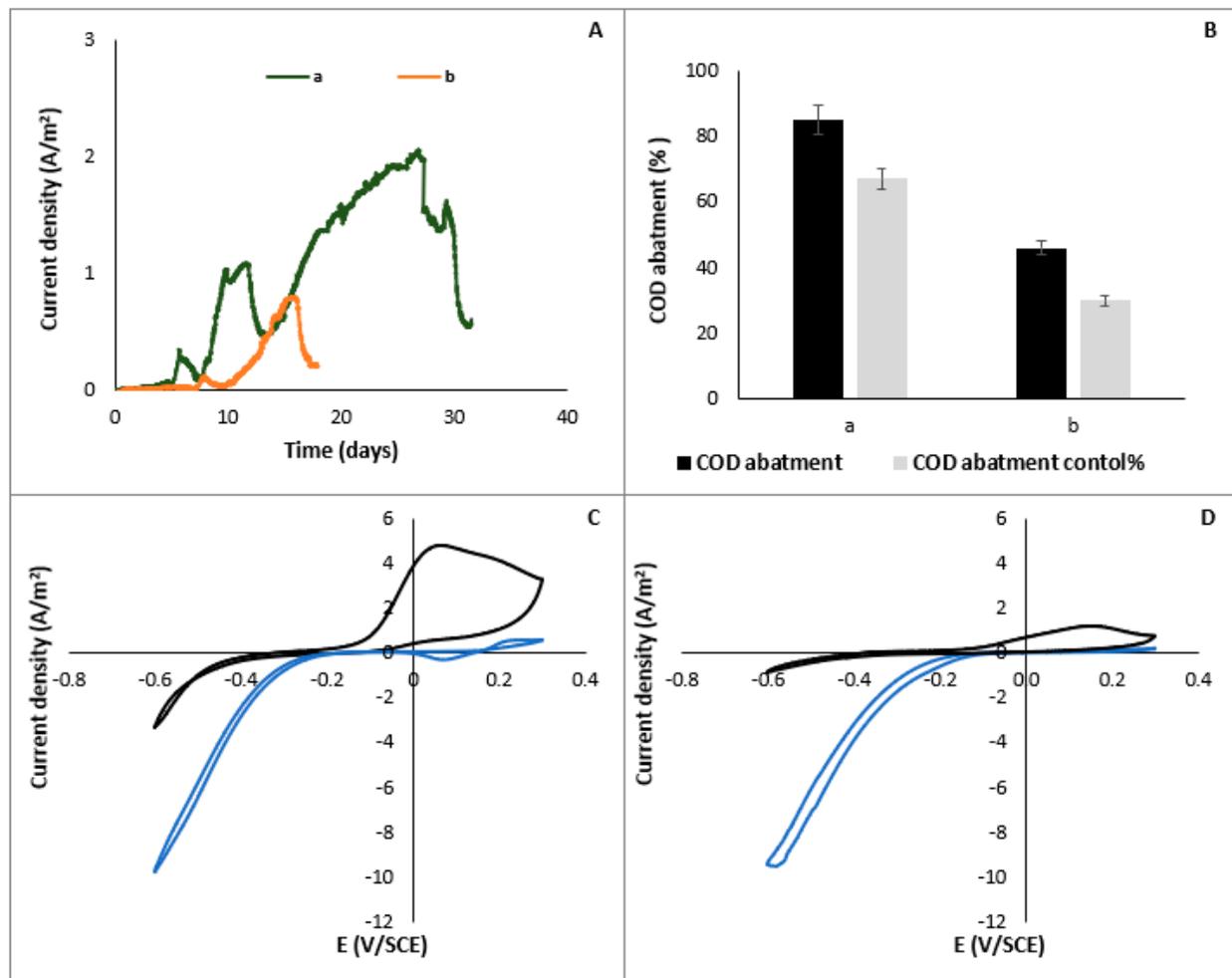


Figure 5. (A) Evolution of the anodic current recorded on the 6 cm² carbon felt working electrode polarized at -0.1 V/SCE in reactor contained RTWW and SCD (a) or SSM as inoculums (b). (B) Decolorization rate achieved by bioremediation or bioelectroremediation. (C) Related cyclic voltammetry CV_{i,SCD} (–), CV_{f,SCD} (–); (D) related cyclic voltammetry CV_{i,SSM} (–) and CV_{f,SSM} (–).

Table 2 shows different decolorization and COD removal rates obtained after treatment of RTWW when SCD or SSM sediments were used as inoculum. A COD removal rate of 85 vs. 30% in control reactor, respectively), and decolorization rates of 88% for TB and 84% for BR were reached when used SCD sediment. The COD removal rate in the control reactor was 67%. However, a low COD removal rate was obtained when used SSM as inoculum (46 vs. 30% in control reactor, respectively), the decolorization rates of TB and BR were 72 and 81%, respectively. Results obtained with RTWW confirm the efficiency of microbial community naturally hosted in the SCD inoculum. Previous studies have shown that the decolorization efficiency of dyes in bioelectrochemical systems was better than that in biological anaerobic reactors [33,34]. Comparing to conventional biological treatment processes, BES could improve the efficiency of the degradation of several pollutants such as volatile organic compounds and azo dyes [6,35]. In fact, these systems have the particularity of coupling microbial metabolisms and electrochemical oxidation–reduction reactions into a single integrated unit [36].

Table 2. COD removal efficiency of TDWW from different reactors SCD and SSM used as inoculums.

	Inoculum	
	SCD	SSM
Input COD (mg/L)	654	950
Output COD (mg/L)	98	514
COD output control (mg/L)	211	682
COD removal rate (%)	85	46
COD removal rate control (%)	67	30
Removal rate of TB (%)	88	72
Removal rate of BR (%)	84	81

The comparison of COD removal rates between the bioelectrochemical reactor and the biological control showed a significant treatment improvement of 18% for the SCD and 37% for the SSM (Figure 5B). According to the literature, biological treatment of textile effluents loaded with azo dyes shows in most studies poor to moderate COD removals ranging from 37 to 63% [37–39]. In fact, the slow microbial process, the incomplete degradation of azo dyes, and the toxicity towards the purifying microorganisms are the three main limitations of conventional biological treatment that significantly limit the COD treatment efficiency [40].

With bioelectrochemical treatment processes, much higher COD abatement rates have been reported. Here, we have demonstrated that the abatement rate can rise significantly to 85%, and other recent work in the literature reports abatements as high as 90% [21,41,42].

Recent studies have confirmed that the capacity of electroactive bacteria (EAB) for extracellular electron transfer (EET) perfectly meets the requirements of the extracellular reduction pathway of azo dyes. Thus, EABs with high EET capacity should also be efficient azo dye reductants due to the role of extracellular cytochrome C (OmcB, OmcC, and OmcE), which are identified as key outer membrane proteins for extracellular dye reduction [43,44]. Compared to the intracellular decolorization common to the conventional biological reactor, extracellular decolorization can be completely catalyzed by these outer membrane proteins directly, thus protecting bacteria from toxin effects by keeping azo dyes and their decolorized products out of the cells, resulting in superior decolorization rates with EAB.

The combination of these two approaches necessarily involves a combination of different reduction pathways: extracellular (by EAB) and intracellular (by bacteria in the anolyte). Indeed, having a polarized electrode for bioelectrocatalyst biofilm formation seems to be an efficient way to boost the degradation of organic matter within an effluent, but also to escape the bacterial toxicity caused by the classical intracellular reduction pathway.

To gain insights into azo dyes degradation by the BES, FTIR was used to reveal possible transformations in their structures. The FTIR spectrum (Figure 6) of the two control azo dyes (control group-TB (a) and control group-BR (b)) revealed that peaks around 3400 cm^{-1} corresponds to $-\text{NH}_2$ of aniline; however, the presence of weak bands at 2900 and 2850 cm^{-1} indicated $-\text{CH}$ stretching. The peak at 1610 cm^{-1} represents the stretching vibration of the $\text{C}=\text{C}$ group on the benzene ring, and the peak at the region from 1400 to 1600 cm^{-1} represents the absorption band of the azo doublebond of TB and BR. The band observed at 1350 cm^{-1} is the characteristic $-\text{CN}$ absorption peak of N atoms linked to the benzene ring. The peak at 1100 cm^{-1} for the BR and 1150 for the TB represents the asymmetric stretching vibrations of sulfonates. The peak at $450\text{--}1000\text{ cm}^{-1}$ represents the out-of-plane bending vibration absorption peak of the benzene ring. Compared with the FTIR spectrum of treated STWW-TB-SCD, TSWW-BR-SCD, RTWW-SCD, STWW-TB-SSM, and RTWW-SSM (Figure 6(c–g)), it is observed that the absorption peak of azo vibrations of wave number at $1400\text{--}1600\text{ cm}^{-1}$ was significantly altered, which argued that the double bond $-\text{N}=\text{N}-$ of TB and BR was depleted [24,45]. Indeed, the degradation of azo dyes starts with the breaking of the azo double bond in the functional chromatic group of azo dyes. Similarly, the two peaks at 1100 and 1150 cm^{-1} had completely disappeared, suggesting the cleavage of the

sulfonic groups. However, the peak around 3450 cm^{-1} remained unchanged, which could be explained by new degradation products containing $-\text{NH}_2$ group that were produced after dye degradation.

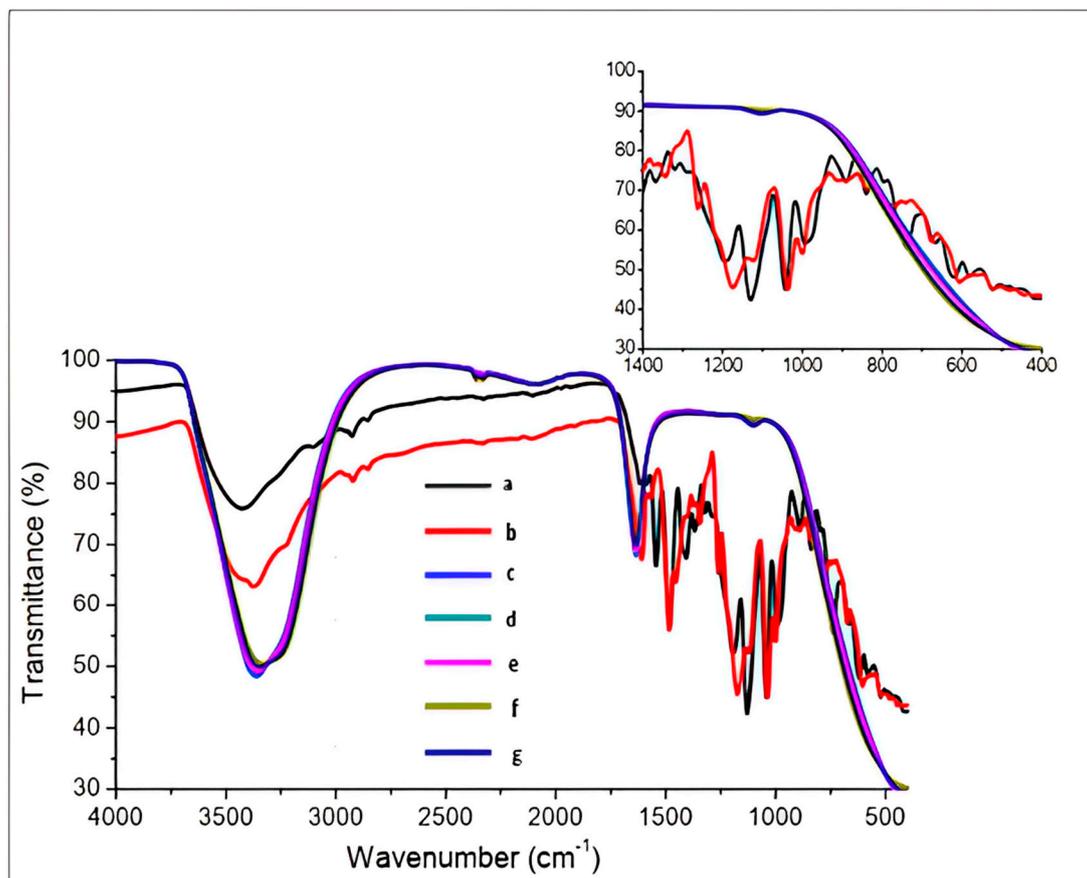


Figure 6. FTIR spectrum analysis of azo dyes TB and BR and their degradation products in STWW and RTWW: (a) control group-TB, (b) control group-BR, (c) STWW-TB-SCD, (d) TSWW-BR-SCD, (e) RTWW-SCD, (f) STWW-TB-SSM, (g) RTWW-SSM.

3.4. Microscopy Analysis of Biofilms Morphology

The adhesion of microorganism cells on the anodic materials was studied by scanning electron microscopy (SEM) after the treatment process (STWW, Figure 7A,B; RTWW, Figure 7C). A diverse community of microorganisms with different structures and morphological forms were detected on the conductive surface, such as *coccus* and *bacillus*. It is important to mention that biofilms obtained when used SCD as inoculum are more colonized than those obtained with SSM inoculum. For micrographs obtained from STWW1 (TB) reactors (Figure 7A), the colonization seems heterogeneous, and the cells are hardly distinguishable, suggesting the presence of very cohesive extracellular polymeric substance (EPS). While for those obtained from STWW2 (BR) reactors (Figure 7B), a thick, fairly homogeneous, and very dense mat of microbial cells was observed. It also seems that above this basal layer there are more isolated clusters, which are about $10\mu\text{m}$ in size and are more anarchic. Biofilms from STWW2 (BR) reactors seem to be more concentrated in EPS because the microbial cells are difficult to differentiate. Micrographs of biofilms from reactors fed with RTWW (Figure 7C) showed a very homogeneous aspect and colonized the entire surface of the carbon fibers. Its thickness is also very regular on the surface. The composition of the biofilm is quite heterogeneous with cells of highly variable size and morphology, and the presence of EPS with non-repeatable patterns. Overall, the SEM images suggest synergistic interactions between different microbial consortia for power generation coupled with textile effluent treatment.

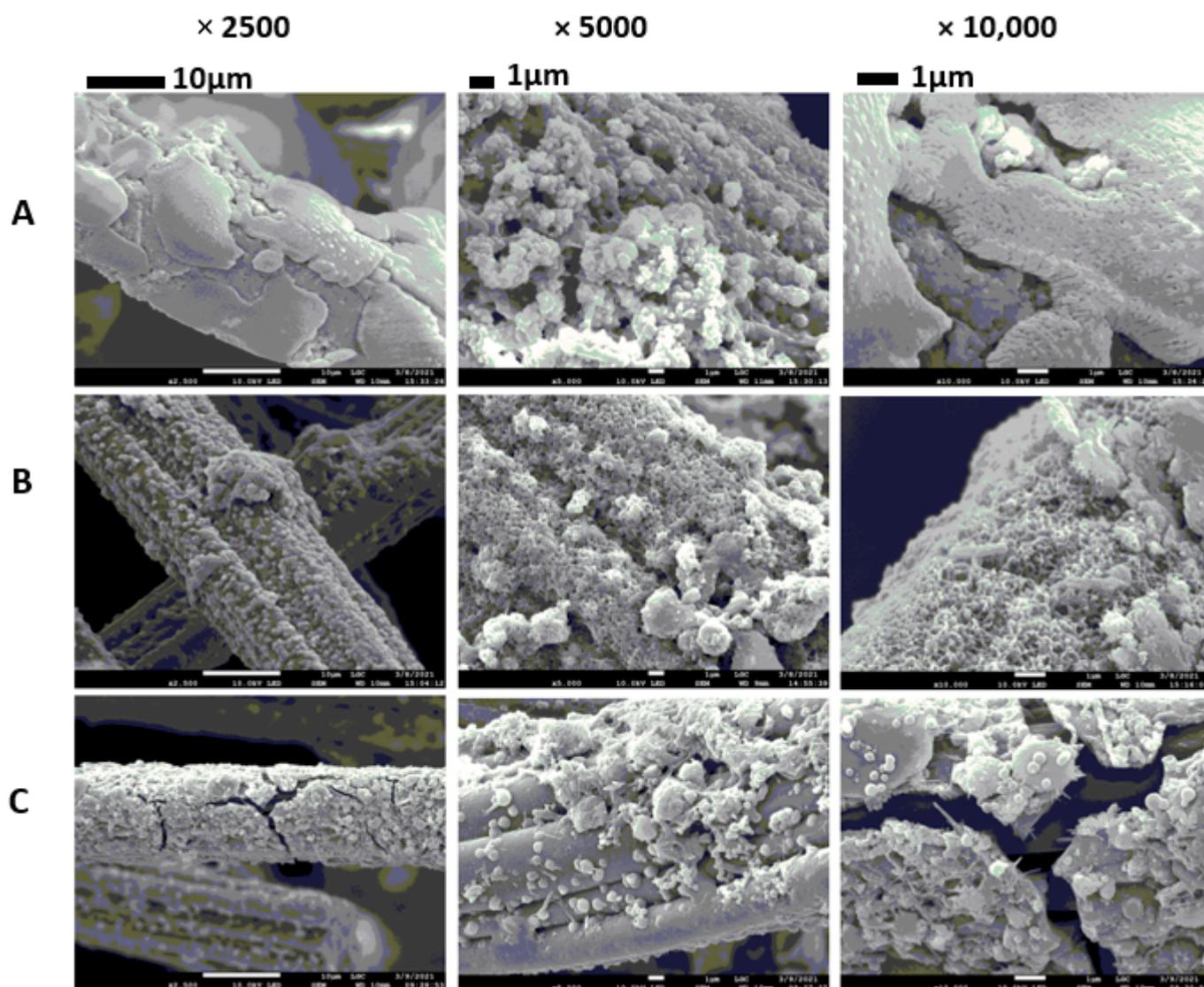


Figure 7. SEM micrographs of carbon felt anode under different magnifications used in BES for textile wastewater treatment and power generation:(A) STWW1 (TB); (B) STWW2 (BR); (C) RTWW.

3.5. Microbial Community Composition

At the end of the electrochemical experiments, different bioanodes named STWW/TB/SSM, STWW/BR/SCD, STWW/TB/SCD, STWW/BR/SSM, RTWW/SCD, and RTWW/SSM were removed from the reactors and subjected to a metagenomic study to probe the variation of bacterial communities within biofilms as a function of the nature of the inoculums (SSM or SCD) and the wastewater (STWW or RTWW). The composition and abundance distribution of each biofilm at the taxonomic levels of phylum and species are shown in Figure 8A,B.

At the phylum level, biofilms were mainly colonized by two phyla: *Proteobacteria* (from 87 to 31%) and *Firmicutes* (from 60 to 31%). *Proteobacteria* were enriched in the pattern of abundance 87% (RTWW/SSM) > 68% (STWW/TB/SCD) > 62% (STWW/TB/SSM) > 54% (STWW/BR/SCD) > 33% (RTWW/SCD) > 31% (STWW/BR/SSM). However, *Firmicutes* were enriched in a different pattern of abundance: 60% (RTWW/SCD) > 50% (RTWW/SSM) > 43% (STWW/BR/SSM) = 43% (STWW/BR/SCD) > 36% (STWW/TB/SSM) > 31% (STWW/TB/SCD). Additionally, the phylum of *Eucyarchaeota* was also enriched in Biofilms STWW/BR/SSM and STWW/BR/SCD collected from reactors, which contained STWW/BR with relative abundances of 24 and 22%, respectively.

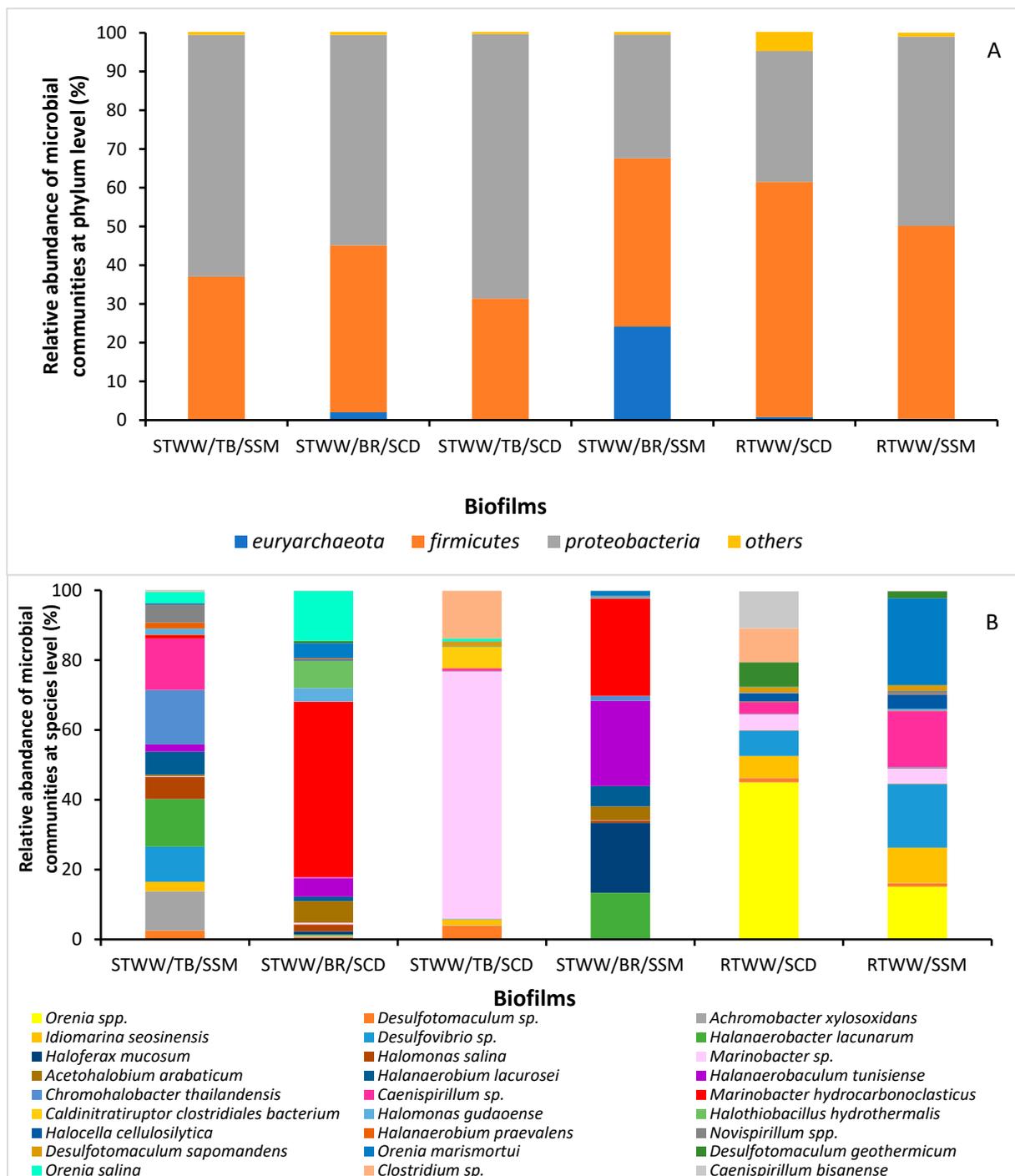


Figure 8. Microbial distribution at (A) phylum level and (B) species level of biofilms STWW/TB/SSM, STWW/BR/SCD, STWW/TB/SCD, STWW/BR/SSM, RTWW/SCD, and RTWW/SSM.

At the species level, biofilms formed in synthetic wastewater contained BR with SSM as inoculum source (STWW/BR/SSM), the most abundant bacteria were *Marinobacter hydrocarbonoclasticus* (27%), *Halanaerobaculum tunisiense* (24%), *Haloferax mucosum* (20%), *Halanaerobacter lacunarum* (13%), *Halanaerobium lacurosei* (6%), *Acetohalobium arabaticum* (4%), and *Orenia marismortui* (1.5%). However, biofilms formed in reactors contained synthetic wastewater containing TB treated with the same sediment (STWW/TB/SSM) were mainly colonized by *Caenispirillum sp.* (15%), *Halanaerobacter lacunarum* (13.6%), *Desulfovibrio sp.* (10%), *Chromohalobacter thailandensis* (15.5%), *Achromobacter xylosoxidans* (11%), *Azospirillum*

oryzae (6%), *Halanaerobium lacurosei* (7%), *Novispirillum* spp. (5%), *Desulfotomaculum* sp. (3%), *Orenia salina* (3%), and *Halanaerobaculum tunisiense* (2%).

Biofilms formed in synthetic wastewater contained TB inoculated with SCD as a source of biocatalyst microorganisms (STWW/TB/SCD) were colonized mainly by the species of *Marinobacter* sp. (71%), *Clostridium* sp. (13.6%), *Caldinitratiruptor clostridiales bacterium* (5%), and *Desulfotolaculum* sp. (4%). While in the case of the synthetic wastewater contained BR treated with the same sediment (STWW/BR/SCD), the biofilms were mainly colonized by *Marinobacter hydrocarbonoclasticus* (50%), *Orenia salina* (14%), *Sporohalobacter* sp. (12%), *Acetohalobium arabaticum* (6%), *Halothiobacillus hydrothermalis* (5%), and *Halanaerobaculum tunisiense* (4%).

These findings clearly revealed an important diversity in the microbial community that colonized the different biofilms. It is important to note that wastewater treated with SCD demonstrated the highest degradation potential and electric current densities; this could be attributed to the microbial community that hosts this sediment mainly being colonized by the genus of *Marinobacter*. This genus has been described as extremely halotolerant with great potential in bioremediation [46]. *Marinobacter hydrocarbonoclasticus* has previously been shown to degrade a wide range of n-alkanes [47] and hydrocarbons [46,48]. Recent genetic studies have shown that its broad metabolic capacity indicates that it should rather be considered as a multipurpose bacterium [49]. Furthermore, the electrogenicity of this strain is increasingly proven. It was described as a major species in a halothermotolerant biofilm designed by Askri et al. [21]. In the same sense Rousseau et al. [50] showed that in biofilm colonized mainly by *Marinobacter* spp. (present strongly in SCD), a very high electron transfer rate was obtained. These data suggest that the *Marinobacter* genus is probably a promising candidate for the design of halotolerant bioanodes.

Regarding experiments carried out with real wastewater and SCD as a source of inoculum, the biofilms were mainly colonized by *Orenia* spp. (45%), *Caenispirillum bisanense* (10.5%), *Clostridium* sp. (10%), *Desulfovibrio* sp. (7%), *Caenispirillum* sp. (3%), *Idiomarina seosinensis* (6%), and *Marinobacter* sp. (5%). In those treated with SSM, biofilms were colonized by *Orenia marismortui* (25%), *Desulfovibrio* sp. (18%), *Caenispirillum* sp. (16%), *Orenia* spp. (15%), *Idiomarina seonensis* (10%), *Marinobacter* sp. (4%), and *Halocella cellulosityca* (4%). Comparing with the bacterial diversity of the communities detected in the biofilms formed in the STWW we notice that the species belonging to *Orenia*, *Idiomarina*, and *Desulfovibrio* genus are only present in the enriched biofilms of the RTWW. According to previous literature, these microorganisms are considered as sulfate-reducing bacteria, and this ability has led them to be used for the remediation of contaminated soils. Additionally, microbial communities belonging to the genus *Desulfovibrio* are well known for their potential to efficiently process azo dyes [51,52]; it is also interesting to note that *Desulfovibrio* possesses an abundance of membrane-bound multiheme cytochromes that are involved in direct extracellular electron transfer [29].

4. Conclusions

In this study, the design of microbial bioanodes, from saline sediments, capable of treating synthetic and real textile wastewater containing azo dyes under different operating conditions were successfully demonstrated. RTWW was used as a substrate to validate the efficiency of the electromicrobial process. A maximum current density of 2.5A/m² and a COD abatement rate of 85% was determined when SCD was used as a source of microorganism. The analysis of the microbial population clearly showed the presence of a mixed microbial community essentially formed by electroactive halotolerant bacteria able to tolerate the conditions of saline wastewaters. These results could lead to possible applications of these bioanodes for bioremediation and energy recovering from textile wastewater containing recalcitrant azo dyes.

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