Biocatalysis and Agricultural Biotechnology 22 (2019) 101376

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: http://www.elsevier.com/locate/bab

A sustainable fungal microbial fuel cell (FMFC) for the bioremediation of acetaminophen (APAP) and its main by-product (PAP) and energy production from biomass

M. Pontié [a,*], E. Jaspard [b], C. Friant [a], J. Kilani [a], A. Fix-Tailler [a], C. Innocent [c], D. Chery [d], S. F. Mbokou [e], A. Somrani [a,f], B. Cagnon [g], P.Y. Pontalier [h]

[a] UBL Universities, University of Angers, Laboratory GEIHP EA 3142, Institut de Biologie en Santé, PBH-IRIS, CHU, 4 Rue Larrey, 49933, Angers Cedex 9, France
[b] UBL Universities, University of Angers, Department of Biology, 2, Bd Lavoisier, 49045, Angers Cedex, France
[c] Institut Européen des Membranes, UMR 5635, ENSCM-UMII-CNRS, Place Eugène Bataillon, 34095, Montpellier, France
[d] Université de Guyane, Centre IRD 228, UMR Espace pour le développement, 274 route de Montabo, BP 90165, 97323 Cayenne Cedex, France
[e] Electrochemistry and Chemistry of Materials, Department of Chemistry, University of Dschang, P.O. Box 67, Dschang, Cameroon
[f] Al Jouf University, College of Sciences and Arts, Physics Sciences Department, Tabarjal, Saudi Arabia
[g] ICMN Interfaces, Confinement, Matériaux et Nanostructures, UMR7374 - Universite d'Orleans–CNRS, 1b rue de la Férollerie, 45071, Orleans Cedex 2, France
[h] Toulouse University, INP-ENSIACET, LCA (Laboratory of Agro-industrial Chemistry), 31030, Toulouse, France

ARTICLE INFO

Keywords:
Fungal microbial fuel cell
S. dehoogii
APAP/PAP/Biomass
Lignin
SEM/AFM

ABSTRACT

A fungal biofilm of *Scedosporium dehoogii* was successfully elaborated and used as a bio anode in a microbial fuel cell device. The cathode was a carbon felt electrochemically modified by electrodeposition of a film of poly-Ni(II) tetrasulphonated phthalocyanine (poly-NiTSPc). The elaborated biofilm, formed by electrodeposition of a suspension of *Scedosporium dehoogii* worked to catalyze acetaminophen (APAP) oxidation in the anode. The optimal potential during the electrodeposition process was found to be 0.8 V *vs.* saturated calomel electrode (SCE) with a thickness of 3.5 ± 0.2 µm. The fabricated fungal microbial fuel cell (FMFC) proved to be an efficient fuel in acetaminophen with highly stable output performances offering a power density of 50 mW m^{-2} under an electromotive force of +550 mV in physiological conditions. The biodegradation of PAP, the main APAP by-product from bacterial biodegradation, was also studied and the optimal resistance from the usual polarization curves was 3.000 Ohm. Finally, the biodegradation of ligno-cellulosic materials such as bagasse, rapeseed, cellulose and lignin by *Scedosporium dehoogii* was successfully tested in Petri dishes. The growth of the fungus using these biomaterials as substrates followed the order: bagasse > rapeseed > cellulose > lignin. The FMFC was tested in the presence of natural lignin from sugarcane bagasse and complete degradation of the lignin by *S. dehoogii* biofilm was achieved with a power density of 16 mW m^{-2}.

1. Introduction

The first recorded occurrence of electrochemical activity between bacterial/fungal (yeast) species and electrodes can be traced back to the early 20th century. It was reported by Potter, 1911), cultures of *Escherichia coli* and *Saccharomyces spp*. produced electricity using platinum macroelectrodes in a battery type setup with sterile media. The story of electromicrobiology gained new interest in 1963, when a NASA space program demonstrated the possibility of recycling and converting human waste to electricity during space flights. In 1991, pioneering work by Habermann and Pommer reported the long-term use of municipal wastewater by a microbial fuel cell (MFC) without malfunction or maintenance. Moreover, this study reported indirect electron transfer (a mechanism of electron transfer allowing specific bacteria to donate electrons) *via* soluble mediators. In 1999, it was discovered that mediators were not essential components within MFC configurations, thus allowing MFCs to be developed without the need for expensive mediators (see the review by Slate et al., 2019).

Since 2002 (Bergel and Féron, 2002), the performances of MFCs have quickly grown and leveled off in 2008 respectively at a few W m^{-2} in laboratory conditions, and at a few mW m^{-2} in natural environments, for a geometrical surface electrode (de Dios et al., 2013). This stimulated

* Corresponding author.
E-mail address: maxime.pontie@univ-angers.fr (M. Pontié).

https://doi.org/10.1016/j.bcab.2019.101376
Received 16 July 2019; Received in revised form 2 October 2019; Accepted 5 October 2019
Available online 7 October 2019

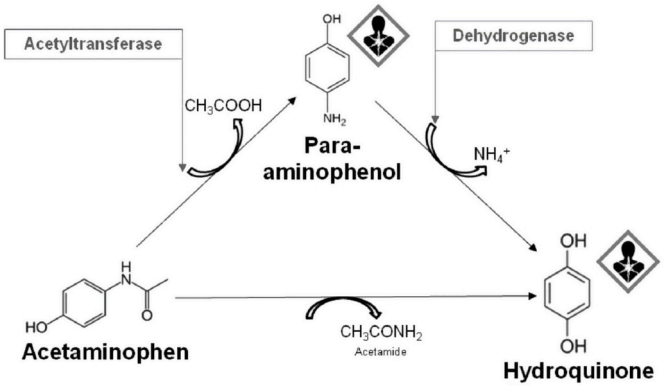


Fig. 1. Illustration of 2 degradation pathways of acetaminophen by bacteria (from Zhang et al., 2013).

increasing interest in electromicrobiology research, which now boasts over a thousand energy-generation-cells reported in the literature (Rahimnejad et al., 2016; Ghasemi et al., 2013). The first commercial prototypes are expected soon (Slate et al., 2019).

Concerning filamentous fungi, the most intensively studied systems are yeast-based MFCs, where direct electron transfer via cytochrome *c* was reported (Hubenova and Mitov, 2015). However, recent findings indicate that other fungi also have redox-active enzymes capable of electrogenic activity in MFC systems (Morath et al., 2012; Rahimnejad et al., 2015). Fungi belonging to the group of white-rot (well-known pharmaceuticals (Marco-Urrea et al., 2009) and wood degraders) were found to have an extracellular oxidative ligninolytic enzymatic system that enables them to degrade lignin (Pollegioni et al., 2015). The enzymes of this system can also degrade various xenobiotic compounds and dyes (Sekrecka-Belniak and Toczyłowska-Maminska, 2018).

Research focusing on energy gain often needed to study their environmental impact (zero waste). Indeed, the presence in treated wastewater of aromatic drug molecules such as diclofenac, ibuprofen, paracetamol or carbamazepine (Langenhoff et al., 2013) and their aromatic by-products (p-aminophenol, hydroquinone …) demonstrates the inefficiency of bacterial treatments of these molecules in conventional wastewater treatment units (Mashkour et al. 2016). All of these molecules may present a carcinogenic, mutagenic and reprotoxic (CMR) hazard, as illustrated in Fig. 1 for APAP.

Many polycyclic aromatic hydrocarbons (PAHs) are also CMR compounds that can be degraded by several fungal enzymes (Kadri et al., 2017).

Scedosporium dehoogii is a filamentous fungus belonging to a complex of five fungi strains named *Scedosporium apiospermum* (Gilgado et al., 2008), living in soil (Alvarez and Sanhueza, 2016) and able to use aliphatic and aromatic hydrocarbons as carbon and energy sources: it can therefore be a good candidate for bioremediation purposes (Blasi et al., 2016). This microorganism is mainly found in polluted waters and contaminated soils (sewage sludge, stagnant water …). Although there are few data about the ecology of this filamentous fungus, it is known that its ecological environment is strongly impacted by human activities and this is true for almost all fungi (Harms et al., 2011). This was shown through its ability to metabolize hydrocarbons (especially aromatics) as a source of carbon and energy (Blasi et al., 2016). This filamentous fungus is a model of bio-indicators for anthropogenic pollution, and also a microorganism of choice for bioremediation, as recently reported (Sekrecka-Belniak and Toczyłowska-Maminska, 2018).

As a typical example, Mbokou et al. (2016a) demonstrated that the biodegradation of APAP by *S. dehoogii* does not lead to the formation of either *p*-aminophenol (PAP) or hydroquinone (HQ). Moreover, the first *S. dehoogii*-based MFC using APAP as fuel was recently developed with a power density of 6.5 mW m^{-2} (Mbokou et al., 2017).

The power density obtained for various fungi/yeast MFCs is presented in Table 1.

Table 1
Examples of some fungi-/yeast-based MFC performances.

Micro-organism	Electron donor	Power density (mW m^{-2})	Reference
Saccharomyces cerevisiae	glucose	155	(Bennetto et al. (1983))
Saccharomyces cerevisiae	synthetic wastewater	283	(Raghavulu et al. (2011))
Saccharomyces cerevisiae	glucose	344	(Christwardana and Kwon (2017))
Arxula adeninivorans	dextrose and glucose	28	(Haslett et al. (2011))
Candida melibiosica	various carbohydrates	60	(Hubenova and Mitov (2010))
Candida sp.	glucose from wastewater	21	Lee et al. (2015)
Hansenula anomala	various carbohydrates	690–2900	Prasad et al. (2007)
Scedosporium dehoogii	APAP	6.5	Mbokou et al. (2017)
Scedosporium dehoogii	APAP	50	**This work**
Scedosporium dehoogii	Lignin	16	**This work**

The present paper reports the results of an investigation into the potential bioremediation of para-aminophenol (PAP) from wastewater and energy production in an MFC comprising an *S. dehoogii* carbon felt anode. The oxidation of APAP and energy production were studied. The feasibility of the biodegradability of PAP by *S. dehoogii* was also explored. Finally, the biodegradability of several ligno-cellulosic materials (bagasse, rapeseed, cellulose, lignin) by *S. dehoogii* was studied in Petri dishes and the natural lignin from sugarcane bagasse was tested for the first time as fuel in a FMFC.

2. Experimental

2.1. Reagents

APAP was purchased from Sigma-Aldrich. A 0.1 M phosphate buffer solution (PBS) of pH 7.4 was used as supporting electrolyte. All other aqueous solutions were prepared from analytical grade chemicals and deionized water with a pH of 6.5, conductivity <1 ??S cm^{-1} and TOC <0.1 mg L^{-1} (Elga Lab Water ultrapure-water system, Purelab-UV-UF, Elga).

2.2. Apparatus and electrochemical measurements

All the electrochemical experiments were performed using a PG580 analyzer (Uniscan Instruments, UK). The electrochemical software used was UiEchem version 3.27 (Uniscan Instruments, Biologic company, France). A conventional three-electrode cell configuration was employed. Working electrodes (0.071 cm^2 of geometric area) as recently published (Prasad et al., 2007) consisted in carbon paste electrodes (CPE) modified by cellulose fibers. The reference electrode was a saturated calomel electrode (SCE). Platinum wire (10 cm length and 4 mm diameter) and grid (1 cm of diameter) were respectively used as the counter electrodes for APAP/PAP/4-hydroxybenzoate (4-HBz) analysis by CPE and biofilm polarization/cathode modification. An aqueous sample was taken once daily from the anode compartment. The residual concentration of APAP was electrochemically determined using square wave voltammetry (SWV) on our homemade modified CPE. For APAP and PAP analyses, the SWV optimized parameters were a frequency of 400 Hz, pulse height of 90 mV, scan increment of 15 mV and initial potential of −0.2 V *vs.* SCE.

Table 2
Radial diameter (in mm) of the mycelium of 4 strains of *S. dehoogii* grown on different carbon sources at 25 °C.

Strains	Source of carbon			
	4-HBz	APAP	HQ	PAP
110350150–01 (1)	21	15	14	14
110354504–01 (2)	18	15	12	14
110350859–01 (3)	20	15	9	11
110350889–01 (4)	17	17	10	7

Strain 1 appeared to be the most effective in metabolizing APAP and its degradation by-products (PAP and HQ), with 4-HBz as the reference. Based on these results, we chose strain 110350150-01(1) for the rest of the experiments.

2.3. Strains and materials

Four *S. dehoogii* strains UA 110350859-01, 110350889-01, 110354504-01 and 110350150-01, isolated from soil samples in the vicinity of Angers (France) were used throughout this study in order to find the best strain to use in the MFC device. The fungi were routinely maintained by weekly passages on a yeast extract-peptone-dextrose (YPD) agar medium containing in g L^{-1}: yeast extract, 5 g; peptone, 5 g; dextrose, 20 g and chloramphenicol, 0.5 g. The ability of *Scedosporium* strains to use APAP, PAP, HQ and 4-hydroxybenzoate (4-HBz) as the sole carbon source was investigated by cultivating the fungi on Scedo-Select III agar plates (Pham et al., 2015) containing each molecule respectively, as follows. Conidia were harvested from 1-week-old cultures at 25–30 °C on YPD medium plates by flooding the agar surface with 15 mL of ultrapure water. The fungal suspension was then filtrated on a 40 µm pore size sterile nylon filter and conidia were pelleted by centrifugation at 4000*g* (5 min at 4 °C). They were resuspended in 10 mL of sterile ultrapure water, and finally enumerated using a hemocytometer. Conidia were then inoculated onto Scedo-Select III agar plates containing either APAP, 4-HBz, PAP or HQ, as carbon sources. For this purpose, stock solutions of these pharmaceuticals (1 g each in 100 mL of ultrapure water) were prepared and sterilized by filtration (0.2 µm pore size sterile membrane). After addition of the carbon source to the culture medium at a final concentration of 0.9 g L^{-1} and inoculation with conidia of *S. dehoogii* (10^4 in 10 µL), the plates were incubated during 1 week at 25 °C. Growth of the fungus was followed by measuring the diameter of the mycelium on the plate every day for 1 week (see Table 2 in the results and discussion section).

Biofilm formation by *S. dehoogii* was obtained by two successive subcultures on the same medium containing 4-HBz instead of glucose. After two weeks of incubation, conidia were harvested from cultures on YPD plates by flooding the agar surface with 15 mL of ultrapure water. The suspension was then filtrated on a 40 µm pore size sterile nylon filter and conidia were pelleted by centrifugation at 4000*g* for 5 min at 4 °C. They were resuspended in 10 mL of sterile ultrapure water. Finally, a suspension of roughly 10^6 spores mL^{-1} was used for the formation of the biofilm on carbon felt.

2.4. Bioanode elaboration

Carbon Felt (CF) (Carbon Lorraine supplier, France) (CF geometrical area = 10 cm^2) was used as the substrate for fungal biofouling deposition. Prior to use, the CF was cleaned successively using a 1 M HCl solution and ultrapure water. Following the usual practice, the CF was then immersed in a 1:1 (volume ratio) mixture of ethanol-water for a few minutes followed by sonication 2 min (47 kHz) in ultrapure water to achieve ethanol rinsing. The CF was finally autoclaved at 120 °C during 15 min before colonization by the fungus.

The CF anode was immersed in a suspension of *S. dehoogii*. The biofilm was then developed by polarization of the CF at −0.15 V *vs.* SCE during one week under sterilized conditions (laminar oven) (Fig. S2, Supplementary Information). The sole source of carbon was 4-HBz at 0.9 g L^{-1} for the formation of the biofilm. As illustrated in Fig. S2, above 90% of the 4-HBz was consumed after 7 days. The initial current density at the potential −0.15 V vs SCE was 175 mA m^{-2} at t = 0 and after 1 week attained the value of 375 mA m^{-2}. The potentiostat used was an electrochemical analyzer VASD 40, connected to a three-electrode system. Carbon felt, SCE and a platinum wire were the working electrode, the reference electrode and the counter electrode, respectively. The homemade cellulose-based CPE was used to follow the consumption of 4-HBz.

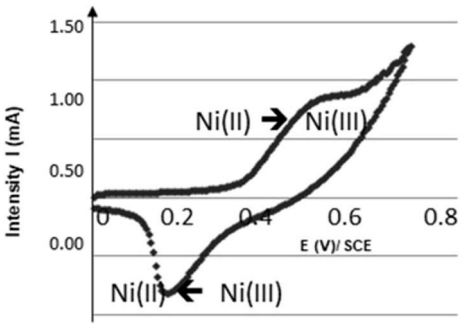


Fig. 2. Cyclic voltammetry of CF/poly-NiTSPc cathode in NaOH 0.1 M.

2.5. Preparation of CPE

The CPE was prepared by thoroughly hand mixing 5 mg of cellulose fibers, 30 mg of silicone oil and 65 mg of graphite powder (analytical grade, ultra F, < 325 mesh, from Alfa) in a mortar, as described elsewhere (Pontie et al., 2017). A portion of the composite mixture was packed into the cylindrical hole of a Teflon® tube equipped with a copper wire serving as electrical contact to maintain contact for electrochemical measurements. The surface exposed to the solution was polished on a weighing paper to give a smooth aspect before use.

2.6. Cathode elaboration

The CF cathode was first electrochemically pretreated under the following conditions: CV between 0.0 and 1.2 V *vs.* SCE in 0.1 M NaOH during 10 cycles (scan rate 0.1 V s^{-1}). CF pretreatment is essential to achieve good reproducibility of the poly-NiTSPc film deposition. The electrochemical deposition of poly-NiTSPc was achieved in 0.1 M NaOH and 2 mM NiTSPc aqueous solution at a fixed potential of +0.8 V *vs.* SCE during 3000 s in order to optimize the poly-NiTSPc matter to be deposited. The effect of poly-NiTSPc electrodeposited on a carbon felt surface has been studied previously (Champavert et al., 2015). Although the electrocatalytic effect of this film on oxygen reduction has not been demonstrated, the presence of the poly-NiTSPc film was useful to reach a larger surface area. In this study, preparation of the poly-NiTSPc film was optimized in terms of its electrodeposition potential.

We then optimized the electrodeposition potential between 0.4 V and 1.2 V (results not shown). No film was observed below 0.6 V and over 1.0 V due to overlap between water oxidation and film electrodeposition. The optimum potential observed was 0.8 V *vs.* SCE. The thickness of the electrodeposited film was estimated at 3.5 ± 0.2 µm from Faraday's law.

The CF zeta potential was measured with a commercialized zetameter apparatus (surPASS3, Anton Paar Company, Austria). The measures were carried out with KCl 10^{-4} M and pH 6.5, for unmodified and modified poly-NiTSPc CF. A zeta potential of −25 mV was obtained for the unmodified as in presence of poly-NiTSPc film the zeta potential value decreased to −49 mV, proving the electrodeposition of poly-NiTSPc on CF, as also proved by the redox system Ni(III)/Ni(II) observed in Fig. 2.

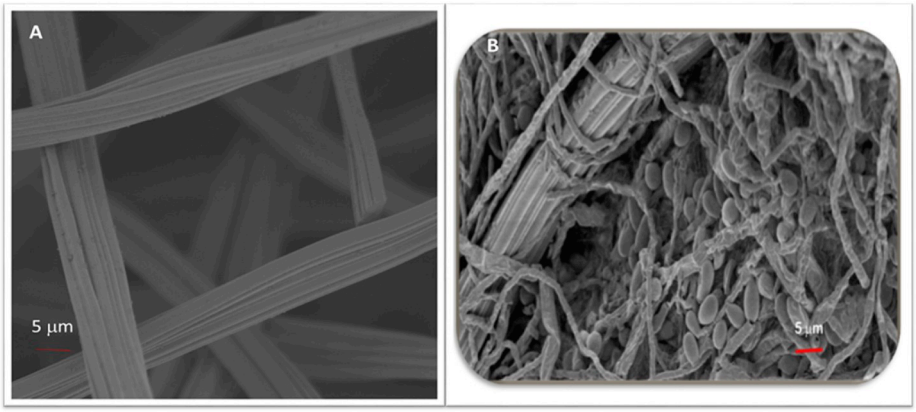


Fig. 3. 2D-FEG-SEM image of unmodified carbon felt (A) and carbon felt covered by a biofilm of *Scedosporium dehoogii* after polarization during 7 days (B).

2.7. Fungal microbial biofuel cell set up

The FMFC set up was divided into two compartments, as recently reported (Mbokou et al., 2017). The compartments were separated by a Nafion 117© membrane which allows protons to cross it from the anode to the cathode chamber. The anode chamber solution consisted of 100 mg L^{-1} APAP in 0.1 M phosphate buffer solution (PBS), pH 7.4. Nafion 117 samples were conditioned in a fridge (4 °C) in NaCl aqueous solution at a concentration of 1 M and fully rinsed with ultrapure water before use in the FMFC.

The solution of the cathode chamber was also PBS. O_2 from air was provided to the cathode chamber before filtration using a 0.45 µm filter. An external resistance of 1 kΩ was connected to the electrodes in order to shuttle electrons from anode to cathode. This external resistance was used to determine the power output during MFC discharge. The MFC started to function when APAP (100 mg L^{-1}) was added in the anode compartment. Residual APAP concentrations in this compartment were measured daily using the SWV technique on the CPE as described above (see section 2.5). All our experiments were carried out at room temperature. In open circuit the electromotive force was measured at +550 mV. During the experiments measuring the power density we estimated that the internal resistance of the MFC was around 5000 Ohm. Furthermore, as an initial control we did not observe any power density in the absence of APAP (or lignin).

2.8. TOC and phenolic compounds (polymeric and monomeric) analyzed in the FMFC

Total organic carbon (TOC) was measured for the experiments conducted in the presence of APAP as the carbon source. The fractional removal of TOC was estimated by measuring the TOC with a TOC-meter-LCSH FA (Shimadzu). TOC was deduced from the values of total carbon (TC) and inorganic carbon (IC). The mobile phase was an acetonitrile/water mixture introduced at a flowrate of 0.8 mL min^{-1} with a 5–80% gradient.

In the case of Lignin as the carbon source in the FMFC, the phenolic composition of the medium was analyzed before and after the experiment according to the Folin-Cioclateu method with 96-well microplates. Each well was fed 20 µL of sample, then 10 µL of commercial Folin-Ciocalteu reagent and 170 µL of Na_2CO_3 alkaline solution at 2.36% were added. The optical density was read after 45 min of reaction at 700 nm with a specific plate reader (BMG-Labtech Spectrostar-Nano). This colorimetric method was used with a calibration curve obtained with different concentrations of gallic acid. The total concentration in phenolic compounds is therefore given in gallic acid equivalent.

The phenolic monomer composition of the medium was characterized by HPLC on an OmniSpher 3 C18 100 x 4.6 column (Agilent Technologies). The gradient was as follows: 91% acidified water (1% acetic acid (v/v)) and 9% acetonitrile for 25 min, from 9 to 90% acetonitrile in 5 min, kept constant for 5 min, then decreased back to 91% acidified water in 5 min; the column was equilibrated for 7 min between runs. The flow rate was 0.5 mL min^{-1}, the injection volume was 10 µL and the column temperature was maintained at 25 °C. The UV detector was set at 280 nm. Concentrations for the calibration curves ranged between 0 and 200 mg L^{-1}. Standard and process samples were diluted in acetonitrile: water at a ratio of 50:50 (v/v) prior to injection. Gallic acid, 4-hydroxybenzoic acid, caffeic acid, syringic acid, vanillic acid (VA), 4-hydroxybenzaldehyde (4HBA), vanillin, *p*-coumaric acid (*p*-CA), syringaldehyde, ferulic acid (FA), sinapic acid and hydroxycinnamic acid were quantified.

2.9. Field emission gun scanning electron microscopy (FEGSEM) and atomic force microscopy (AFM) characterizations

The FEGSEM equipment used was a LEO 1530 apparatus (SCIAM, Angers University, France) at a 3 keV acceleration voltage to minimize the irradiation damage and obtain a resolution of a few nanometers. The topography 3SD image of the biofilm deposited on the CF was obtained using an Atomic Force Microscopy set-up (Nanoscope III from Bruker, Germany). Samples were attached to steel discs and were then recovered by an ultra-thin layer of Pt (4 nm) deposited by evaporation under vacuum (BAL-TEC MED 020 Balzers Lichentstein apparatus) for FEG-SEM observations.

2.10. Ligno-cellulosic matter

Bagasse from sugar cane and lignin were provided by the laboratory LCA (Toulouse, ENSIACET, France). Rapeseed was obtained from ICMN (Orleans, France). Cellulose powder was purchased from Fluka Company. Growth of *S. dehoogii* was followed by measuring the diameter of the mycelium on the plate daily during 1 week, as detailed in section 2.3.

3. Results and discussion

3.1. Comparison of the growth of different S. dehoogii strains in the presence of different aromatic molecules

We compared the radial diameter growth (Fig. S1A) on agar plates of four strains of *S. dehoogii* in the presence of different carbon sources by measuring the diameter of the mycelium during one week (Table 2).

3.2. Morphological and topographical characterizations of the fungal bioanode

The surface morphology of the carbon felt was observed (Fig. 3) using FEGSEM apparatus. This revealed the formation of a biofilm.

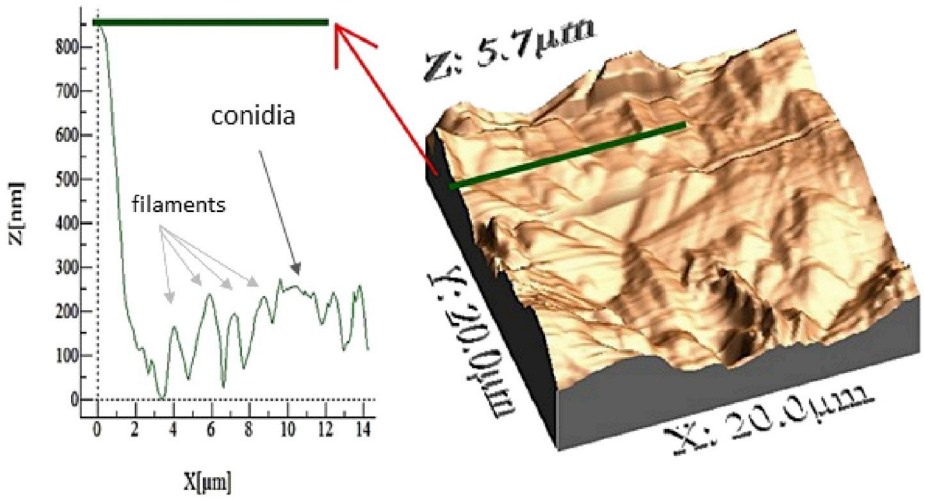
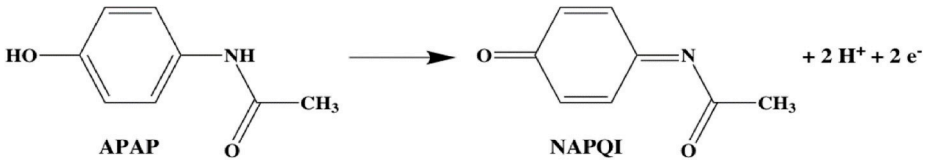


Fig. 4. AFM 3D-image of the *S. dehoogii* biofilm developed on the carbon felt.



Scheme 1. Electrochemical oxidation of APAP.

$$C_8H_9O_2N + \tfrac{37}{2}O_2 \rightarrow 8CO_2 + \tfrac{9}{2}H_2O + \tfrac{1}{2}N_2$$

Scheme 2. APAP combustion reaction (hypothetical) in the FMFC.

After 1 week of polarization, conidia and filaments of the fungus became visible (center - right of Fig. 3). A CF fiber of 12 µm diameter was observed top left.

To complete FEGSEM observations, the topographical characterization of the biofilm was drawn in 3D (Fig. 4). A zoom on the green line in Fig. 7 enabled measurement of the diameter of the filaments (around 2 µm) and that of conidia (around 5 µm).

Figs. 3 and 4 are complementary illustrations of the presence of the *S. dehoogii* biofilm on the CF anode (see also Fig. S2B for a macroscopic view of the bioanode).

3.3. Proving the connection of S. dehoogii to CF

One of the technological barriers often encountered by bio-electrochemists is the connection of the microorganisms to the electrodes, which does not necessarily operate directly. We studied the kinetics of electron transfer for the redox system NAPQI/APAP (NAPQI, N-acetyl-*p*-benzoquinone imine Scheme 1) in order to compare the unmodified CF (control, Fig. 5) and that covered by the *S. dehoogii* biofilm (CF colonized by *S. dehoogii*) (see Scheme 2).

Fig. 5 shows that in the case of the NAPQI/APAP redox system (Scheme 1), the kinetics of electron transfer of *Scedosporium dehoogii* coated CF is faster than that on the unmodified one: a decrement of 500 mV in ΔEp was observed with the biofilm vs no biofilm (see Fig. 5B). At the same time, the current density of the APAP oxidation signal on the colonized CF increased by a factor of 2.5. These results demonstrate the

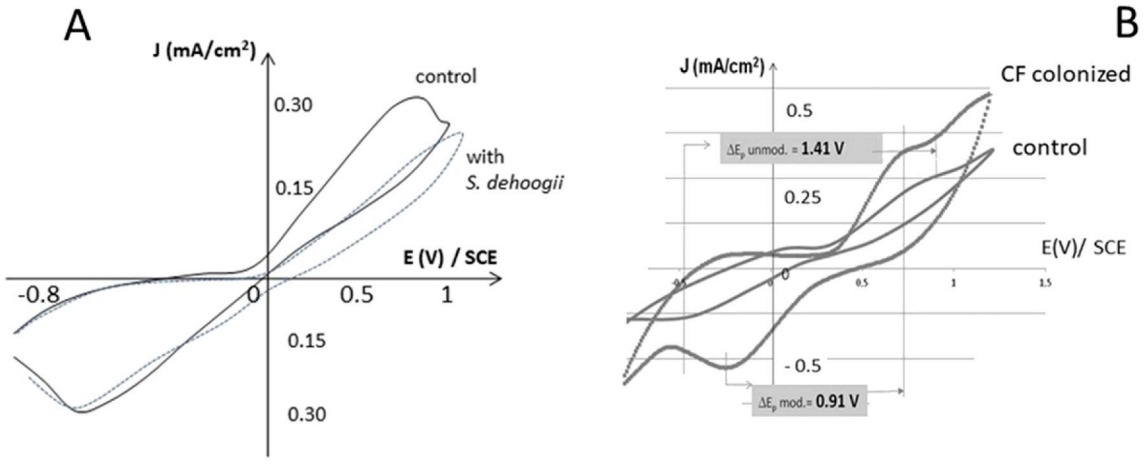


Fig. 5. Cyclic voltammograms of the carbon felt colonized or not, at a scanning rate of 200 mV s^{-1}, in the presence of APAP 50 mg L^{-1} in PBS: **(A)** Non-colonized CF (control) without and with a suspension of *S. dehoogii*; **(B)** CF colonized or not (control) by *S. dehoogii*.

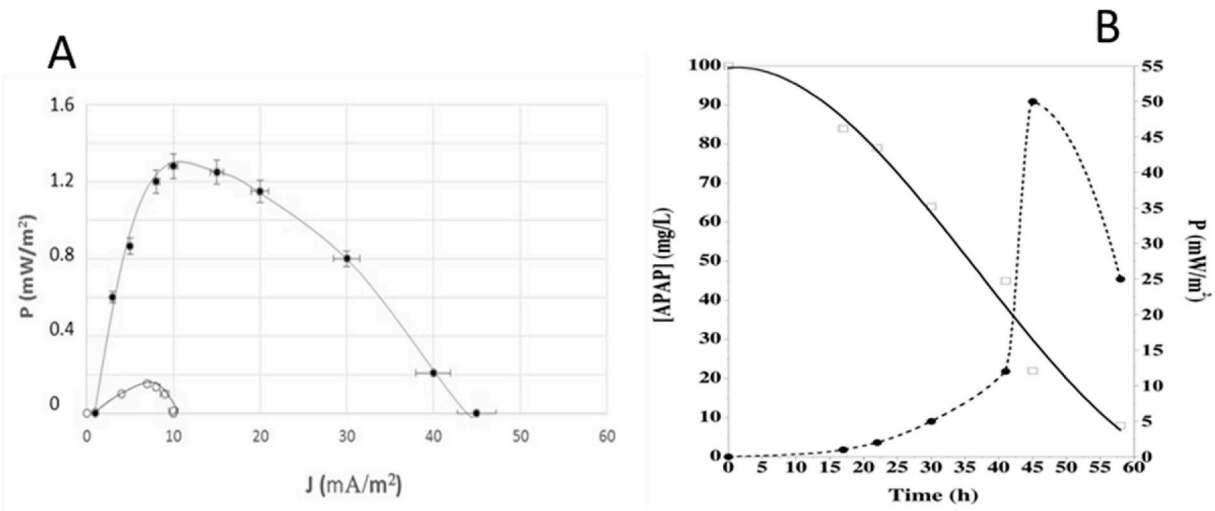


Fig. 6. **(A)** Evolution of the power density *vs.* current density; **(B)** Kinetic of APAP concentration and power density in the fungal MFC. Legend: ●with biofilm; ○ without biofilm.

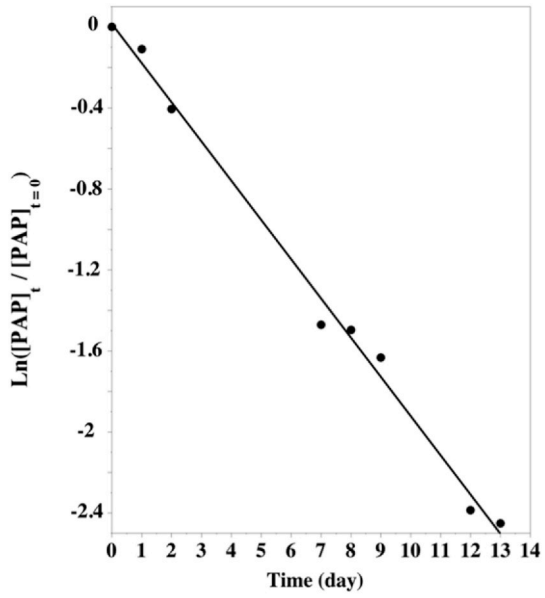


Fig. 7. Kinetic of the biodegradation of PAP by *S. dehoogii* at 25 °C.

ability of the *S. dehoogii* biofilm to electrocatalyze the oxidation of APAP.

It shows for the first time a good connection between the fungus *S. dehoogii* and the anode material. It remains to be elucidated whether it is an external enzyme or an internal process that is involved in this connection.

3.4. FMFC performances

The electrons generated by *S. dehoogii* from APAP are directly transferred to the anode. Then, they flow to the cathode through a conductive material containing a resistor. Electrons reaching the cathode combine with protons diffusing from the anode through the proton exchange and oxygen provided by air, thereby resulting in the formation of water. When electrons flow from the anode to the cathode, they generate current and voltage to make direct energy. Fig. 6 shows the degradation kinetics of APAP along with the power density generated by the MFC.

Fig. 6A shows the polarization curves of the FMFC without and with biofilm. In both cases an optimal resistance was obtained and found to be around 3000 Ohm at the maximum power density. In Fig. 6B, the APAP concentration started to decrease along with the growth of the fungal biofilm on the anode. This means that APAP has been degraded by fungi and used as a carbon and energy source.

The power density delivered by the FMFC increased gradually after APAP addition, indicating the absence of a transitory state (no adaptation period) of *S. dehoogii* (*i.e.*, the biofilm) to the drug. After roughly 40 h, the power density increased to a maximum value of 50 mW m^{-2} while at the same time the APAP concentration decreased. So we observed as elsewhere that APAP decreases when the power decreases.

The shape of the curve is identical to that obtained for the degradation kinetic of another pharmaceutical (*i.e.*, naproxen) by the fungus *Phanerochaete chrysosporium*: degradation percentages were 9% and 83% after 2h and 23 h of fungus action, respectively (Rodarte-Morales et al., 2012).

Furthermore, TOC measured after 7 days in the anode compartment was found to be 22 mg L^{-1} when the initial APAP concentration was 100 mg L^{-1}. No by-products such as PAP or HQ were detected (results not shown).

Then, under the hypothesis of a complete combustion of APAP, the fuel cell reaction can be written:

$$C_8H_9O_2N + \frac{37}{2} O_2 \rightarrow 8CO_2 + \frac{9}{2} H_2O + \frac{1}{2} N_2$$

Then, in a the complete combustion of APAP 37 e−per mol. are delivered as in the case of glucose only 24 e−are released.

Comparing our results with those reported in Table 1, we can observe a higher power density than that found by other authors. This can be attributed to the fact that the biofilm was developed only at the external surface for the CF electrode. In the present work, however, the power density was improved (Champavert et al., 2015) by optimizing the electrodeposition of phthalocyanine dedicated to the oxygen reduction reaction.

3.5. PAP biodegradation studies

The consumption of PAP by *Scedosporium dehoogii* was monitored by our homemade CPE (Fig. 7).

This confirmed the results reported in Table 2, showing that *S. dehoogii* is able to use PAP as a source of carbon in Erlenmeyer flasks. The kinetic follows a process of pseudo-order 1 with a kinetic constant of 0.19 day^{-1}, and a half-life of 3.3 days. This results are closer to those obtained by Mbokou (Mbokou et al., 2017) for APAP biodegradation in

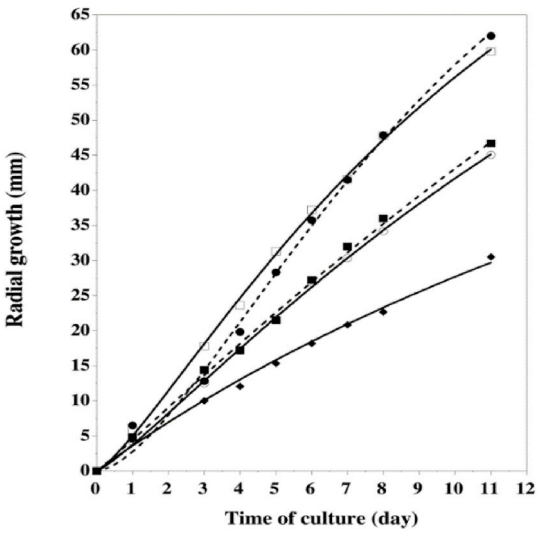


Fig. 8. Radial growth on different carbon sources: (●) bagasse; (□) rapeseed, (○) cellulose, (◆) lignin, (■) PDA, potato dextran agar, is the reference).

the same FMFC set-up, where a kinetic constant of 0.11 day^{-1} and half-life of 6.3 days were found.

3.6. Plant biomass as the source of carbon for Scedosporium dehoogii

Wastewaters usually contain very low concentrations of pharmaceutical compounds (a few μmol L^{-1} or less) and cannot allow our FMFC to attain a very high power density. Therefore, we decided to test a cellulose-based source of carbon from ligno-cellulosic materials (LCM) as a new source of fuel. LCM is mainly composed of cellulose, hemicellulose and lignin. They constitute the most important source of organic carbon on earth and account for nearly all carbon sequestered annually in plant matter (Mbokou et al., 2016b). Lignins are complex nonlinear polymers, whose precursor is phenylalanine (Davin et al., 2008). In particular, lignins are a major source of aromatic carbon on Earth, where it accounts for nearly 30% of carbon sequestered in plant biomass. There is a great diversity of lignins. Plant polymers thus constitute very abundant energy sources whose bioremediation reduces their carbon footprint. Numerous fungi are known to degrade LCM (for example, *Scedosporium apiospermum*, *Pleurotus ostreatus*, *Phanerochaete chrysosporium* …) because they possess lignin-modifying enzymes (Morales et al., 2017).

Fig. 8 shows the growth of *S. dehoogii* in a Petri dish with PDA as substrate in the presence of different plant biomasses: bagasse, rapeseed, cellulose and lignin.

The results show for the first time that *Scedosporium dehoogii* can metabolize different plant biomasses as a source of carbon and energy. As shown in Fig. 8, the rate of growth of the fungus decreased following the order: bagasse - rapeseed > cellulose > lignin.

Based on these encouraging results, we explored the feasibility of lignin as biofuel in our FMFC. In fact, lignocellulosic or second biorefineries transform agricultural by-products or forest biomass into energy (i.e. thermal), various chemicals and also materials (Oriez et al. 2019).

In the last part of our work, we therefore tested for the first time the biodegradability of sugarcane bagasse lignin in our FMFC device.

3.7. FMFC performances with lignin

The fabricated FMFC started functioning when lignin 1 $g.L^{-1}$ (first injection) was introduced into the anode compartment.

Residual lignin species such as monolignols were not detected after 10 days residence time in the anode compartment of the FMFC. A decolorization was observed as illustrated in Fig. 9A. The analysis of the medium with lignin showed that it had an initial concentration of 1.84 g GAE (Gallic acid equivalent)/L. At the end of the experiment, the concentration was only 0.24 g GAE/L, meaning that 87% of the lignin had been removed. HPLC analysis did not show any monomeric compounds, indicating that the degradation of lignin molecules was complete and did not lead to the production of small molecules. It can be assumed that lignin was used as the source of carbon and completely degraded by the strain, which can explain the sharp decrease in the electric production, after 200 h, due to the lack of the carbon source.

The power density generated by the FMFC in the presence of lignin as a function of time was monitored, as illustrated in Fig. 9B. It shows a maximum power density of 16 mW m^{-2} for a time of 200h.

4. Conclusions and perspectives

It has been demonstrated for the first time that a biofilm of *Scedosporium dehoogii* developed by polarization at −0.15 V vs. SCE during one week established a connection to a CF bioanode. The cathode of our fungal MFC presented an optimum potential of 0.8 V vs. SCE for 3.5 ± 0.2 μm thickness of poly-NiTSPc film. We have also shown that APAP is an efficient fuel for this FMFC. Stable output performances were obtained giving a power density of 50 mW m^{-2} for an EMF of 550 mV.

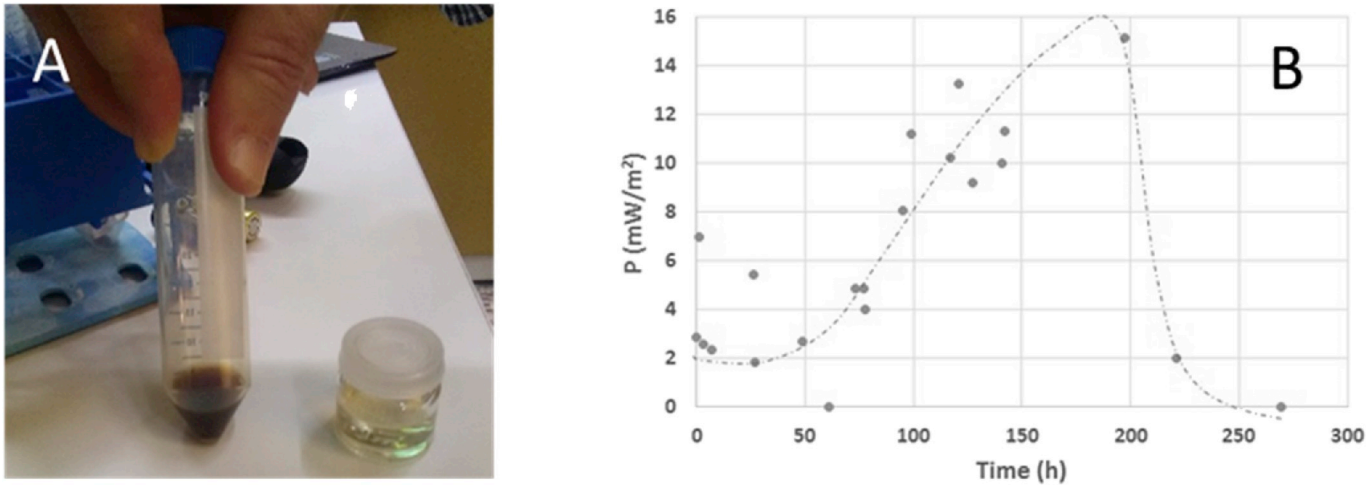


Fig. 9. A- Lignin alkaline solution (brown color) (day 0 in the FMFC) and lignin decolorized (day 10 in the FMFC); **B**- Power density vs time in presence of lignin as source of C. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Furthermore, the biodegradation of PAP, the main APAP by-product from bacterial biodegradation was studied and *Scedosporium dehoogii* was able to use PAP as the sole source of carbon and energy.

Lignocellulosic materials were tested for their ability to be potential carbon sources in the FMFC device. The rate of growth of the fungus decreased following the order: bagasse ~ rapeseed > cellulose > lignin.

The results obtained from the biodegradation of APAP, PAP and the plant materials by *Scedosporium dehoogii* are promising and can be a way to valorize LCM in energy production. The power density delivered by the fungal bioanode is considered better, a fact making *Scedosporium dehoogii* a great alternative for the development of novel FMFCs with sustainable carbon sources.

In the near future, we will conduct first experiments with other fungi as *Scedosporium dehoogii* because very recent results demonstrated its previously suspected pathogenicity for human beings.

The goal will be to achieve a better characterization of the enzymes involved in the degradation processes and to increase the power density developed by our FMFC.

To conclude we have often observed biofouling on the surface of the Nafion© membrane on the side in contact with the fungal biofilm, antifouling strategies should be developed and tested and/or development of a new proton exchange membranes under low cost and dedicated to its application in microbial fuel cells.

Acknowledgments

The authors wish to thank R. MALLET for recording FEGSEM/AFM images at the microscopy department (SCIAM) of the University of Angers (France). We also thank the University of Angers for funding allocated to Dr. Serge MBOKOU FOUKMENIOK for his 4-month internship in France. Many thanks to Julia B. RAZAFIMANDIMBY for providing the strain of *Scedosporium dehoogii*. We are very thankful to the LCA lab (ENSIACET, Toulouse, France) for providing the biomass. Thanks also to LE PALAIS DE LA DECOUVERTE DE PARIS (France), especially to Romain ATTAL who gave us the opportunity to exhibit to the public our work on FMFC during 2 months (Oct. 3rd to Nov. 25th 2018).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101376.

References

Potter, M.C., 1911. Electrical effects accompanying the decomposition of organic compounds. Proc. R. Soc. Lond. B 84, 260–276.

Alvarez, E., Sanhueza, C., 2016. New record of *Scedosporium dehoogii* from Chile: phylogeny and susceptibility profiles to classic and novel putative antifungal agents. Rev. Iberoam. De. Micol. 33, 224–229.

Bennetto, H.P., Stirling, J.L., Tanaka, K., Vega, C.A., 1983. Anodic reactions in microbial fuel cells. Biotechnol. Bioeng. 25, 559–568. https://doi.org/10.1002/bit.260250219.

Bergel, A., Féron, D., 2002. French Patent. CNRS 6 August 2002, (FR 02/100009).

Blasi, B., Polynter, C., Rudavsky, T., 2016. Pathogenic yet environmentally friendly? Black fungal candidates for bioremediation of pollutants. Geomicrobiol. J. 33, 308–317 and references therein. https://www.tandfonline.com/doi/full/10.1080/01490451.2015.1052118.

Champavert, J., Ben Rejeb, S., Innocent, C., Pontié, M., 2015. Microbial fuel cell based on Ni-tetra sulfonated phthalocyanine cathode and graphene modified bioanode. J. Electroanal. Chem. 757, 270–276. https://doi.org/10.1016/j.jelechem.2015.09.012.

Christwardana, M., Kwon, Y., 2017. Yeast and carbon nanotube based biocatalyst developed by synergetic effects of covalent bonding and hydrophobic interaction for performance enhancement of membraneless microbial fuel cell. Bioresour. Technol. 225, 175–182. https://doi.org/10.1016/j.biortech.2016.11.051.

Davin, L.B., Jourdes, M., Patten, A.M., Kim, K.W., Vassao, D.G., Lewis, N.G., 2008. Dissection of lignin macromolecular configuration and assembly: comparison to related biochemical processes in allyl/propenyl phenol and lignan biosynthesis. Nat. Prod. Rep. 25, 1015–1090.

de Dios, M.A.F., del Campo, A.G., Fernandez, F.J., Rodrigo, M., Pazos, M., Sanroman, M. A., 2013. Bacterial–fungal interactions enhance power generation in microbial fuel cells and drive dye decolorization by an ex situ and in situ electro-Fenton process. Bioresour. Technol. 148, 39–46.

Ghasemi, M., Wan Daud, W.R., Ismail, M., Rahimnejad, M., Fauzi, A., Leong, X., Miskan, M., Ben Liew, K., 2013. Effect of pre-treatment and biofouling of proton exchange membrane on microbial fuel cell performance. Int. J. Hydrogen Energy 38, 5480–5484.

Gilgado, F., Cano, J., Gené, J., Sutton, D.A., Guarro, J., 2008. Molecular and phenotypic data supporting distinct species statuses for *Scedosporium apiospermum* and *Pseudallescheria boydii* and the proposed new species *Scedosporium dehoogii*. J. Clin. Microbiol. 46, 766–771.

Habermann, W., Pommer, E.–H., 1991. Biological fuel cell with sulphide storage capacity. Appl. Microbial. Biotechnol. 35, 128–133.

Harms, H., Schlosser, D., Wick, L.Y., 2011. Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. Nat. Rev. Microbiol. 9, 177–192.

Haslett, N.D., Rawson, F.J., Barriere, F., Kunze, G., Pasco, N., Gooneratne, R., Baronian, K.H.R., 2011. Characterization of yeast microbial fuel cell with the yeast *Arxula adeninivorans* as the biocatalyst. Biosens. Bioelectron. 26, 3742–3747. https://doi.org/10.1016/j.bios.2011.02.011.

Hubenova, Y., Mitov, M., 2010. Potential application of *Candida melibiosica* in biofuel cells. Bioelectrochemistry 78, 56–61. https://doi.org/10.1016/j.bioelechem.2009.07.005.

Hubenova, Y., Mitov, M., 2015. Extracellular Electron transfer in yeast-based biofuel cells: a review. Bioelectrochemistry 106, 177–185. https://doi.org/10.1016/j.bioelechem.2015.04.001.

Kadri, T., Rouissi, T., Kaur Brar, S., Cledon, M., Sarma, S., Verma, M., 2017. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: a review. J. Environ. Sci. 51, 52–74.

Langenhoff, A., Inderfurth, N., Veuskens, T., Schraa, G., Blokland, M., Kujawa-Roeleveld, K., Rijnaarts, H., 2013. Microbial removal of the pharmaceutical compounds Ibuprofen and diclofenac from wastewater. BioMed Res. Int. 2013, 325806.

Lee, Y.-Y., Kim, T.G., Cho, K.-S., 2015. Isolation and characterization of a novel electricity-producing yeast, *Candida sp.* IR11. Bioresour. Technol. 192, 556–563. https://doi.org/10.1016/j.biortech.2015.06.038.

Marco-Urrea, E., Pérez-Trujillo, M., Vicent, T., Caminal, G., 2009. Ability of white-rot fungi to remove selected pharmaceuticals and identification of degradation products of ibuprofen by *Trametes versicolor*. Chemosphere 74, 765–772.

Mashkour, M., Rahimnejad, M., Mashkour, M., 2016. Bacterial cellulose-polyaniline nano-biocomposite: a porous media hydrogel bioanode enhancing the performance of microbial fuel cell. J. Power Sources 325, 322–328.

Mbokou, F.S., Pontié, M., Razafimandimby, B., Bouchara, J.-P., Njanja, E., Tonle Kenfack, I., 2016. Evaluation of the degradation of acetaminophen by the filamentous fungus *Scedosporium dehoogii* using carbon-based modified electrodes. Anal. Bioanal. Chem. 408, 5895–5903. https://doi.org/10.1007/s00216-016-9704-8.

Mbokou, F.S., Pontié, M., Bouchara, J.-P., Tchieno, M.M.F., Njanja, E., Mogni, A., Pontalier, Y.P., Tonle, K.I., 2016. Electroanalytical performance of a carbon paste electrode modified by coffee husks for the quantification of acetaminophen in quality control of commercialized pharmaceutical tablets. Int. J. Electrochem. 2016 https://doi.org/10.1155/2016/1953278. Article ID 1953278.

Mbokou, F.S., Kenfack Tonle, I., Pontié, M., 2017. Development of a novel hybrid biofuel cell type APAP/O_2 based on a fungal bioanode with a *Scedosporium dehoogii* biofilm. J. Appl. Electrochem. 47, 273–280. https://doi.org/10.1007/s10800-016-1030-5.

Morales, L.T., Gonzalez-Garcia, L.N., Orozco, M.C., Restrepo, S., Vives, M.J., 2017. The genomic study of an environmental isolate of *Scedosporium apiospermum* shows its metabolic potential to degrade hydrocarbons. Stand. Genom. Sci. 12, 71.

Morath, U.S., Hung, R., Bennett, W.J., 2012. Fungal volatile organic compounds: a review with emphasis on their biotechnological potential. Fungal Biol.Rev. 26, 73–83.

Oriez, V., Peydecastaing, J., Pontalier, P.I., 2019. Separation of sugarcane bagasse mild alkaline extract components by ultrafiltration-membrane screening and effect of filtration parameters. Process Biochem. 78, 91–99. https://doi.org/10.1016/j.procbio.2019.01.006.

Pham, T., Giraud, S., Schuliar, G., Rougeron, A., Bouchara, J.-P., 2015. Scedo-Select III: a new semi-selective culture medium for detection of the *Scedosporium apiospermum* species complex. Med. Mycol. 53, 512–519.

Pollegioni, L., Tonin, F., Rosini, E., 2015. Lignin-degrading enzymes. FEBS J. 282, 1190–1213.

Pontie, M., Mbokou, S., Bouchara, J.-P., Razafimandimby, B.J., Pontalier, P.Y., 2017. Paracetamol sensitive cellulose-based electrochemical sensors. J. Renew. Mater. 6, 242–250. https://doi.org/10.7569/JRM.2017.634169.

Prasad, D., Arun, S., Murugesan, M., Padmanaban, S., Satyanarayanan, R.S., Berchmans, S., Yegnaraman, V., 2007. Direct electron transfer with yeast cells and construction of a mediatorless microbial fuel cell. Biosens. Bioelectron. 22, 2604–2610. https://doi.org/10.1016/j.bios.2006.10.028.

Raghavulu, S.V., Goud, R.K., Sarma, P.N., Mohan, S.V., 2011. *Saccharomyces cerevisiae* as anodic biocatalyst for power generation in biofuel cell: influence of redox condition and substrate load. Bioresour. Technol. 102 (3), 2751–2757.

Rahimnejad, M., Adhami, A., Darvari, S., Zirepour, A., Oh, S.E., 2015. Microbial fuel cell as new technology for bioelectricity generation: a review. Alexandria Eng. J. 54, 745–756.

Rahimnejad, M., Ghasemi, M., Najafpour, D.G., Ismail, M., Mohammad, W.A., Ghoreyshi, A.A., Sedky, H.A., 2016. Synthesis, characterization and application studies of self-made Fe3O4/PES nanocomposite membranes in microbial fuel cell. Electrochim. Acta 85, 700–706.

Rodarte-Morales, A.I., Feijoo, G., Moreira, M.T., Lema, J.M., 2012. Biotransformation of three pharmaceutical active compounds by the fungus *Phanerochaete chrysosporium* in a fed batch stirred reactor under air and oxygen supply. Biodegradation 23, 145–156.

Sekrecka-Belniak, A., Toczyłowska-Maminska, R., 2018. Fungi-Based Microbial fuel cells. Energies 11, 2827–2845. https://doi.org/10.3390/en11102827.

Slate, A.J., Whitehead, K.A., Brownson, D.A.C., Banks, C.E., 2019. Microbial fuel cells: an overview of current technology. Renew. Sustain. Energy Rev. 101, 60–81. https://doi.org/10.1016/j.rser.2018.09.044 and references therein.

Zhang, L., Hu, J., Zhu, R., Zhou, Q., Chen, J., 2013. Degradation of paracetamol by pure bacterial cultures and their microbial consortium. Appl. Microbiol. Biotechnol. 97, 3687–3698. https://doi.org/10.1007/s00253-012-4170-5.