

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

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1 A sustainable Fungal Microbial Fuel Cell (FMFC) for

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26 ABSTRACT: A fungal biofilm of Scedosporium dehoogii was successfully elaborated and used as a 27 bio anode in a microbial fuel cell device. The cathode was a carbon felt electrochemically modified 28 by electrodeposition of a film of poly-Ni(II) tetrasulphonated phthalocyanine (poly-NiTSPc). The 29 elaborated biofilm, formed by electrodeposition of a suspension of Scedosporium dehoogii worked to 30 catalyze acetaminophen (APAP) oxidation in the anode. The optimal potential during the 31 electrodeposition process was found to be 0.8 V vs. saturated calomel electrode (SCE) with a 32 thickness of 3.5±0.2 µm. The fabricated fungal microbial fuel cell (FMFC) proved to be an efficient 33 fuel in acetaminophen with highly stable output performances offering a power density of 50 mW 34 m⁻² under an electromotive force of +550 mV in physiological conditions. The biodegradation of 35 PAP, the main APAP by-product from bacterial biodegradation, was also studied and the optimal 36 resistance from the usual polarization curves was 3.000 Ohm. Finally, the biodegradation of 37 ligno-cellulosic materials such as bagasse, rapeseed, cellulose and lignin by Scedosporium dehoogii 38 was successfully tested in Petri dishes. The growth of the fungus using these biomaterials as 39 substrates followed the order: bagasse > rapeseed > cellulose > lignin. The FMFC was tested in the 40 presence of natural lignin from sugarcane bagasse and complete degradation of the lignin by S. 41 dehoogii biofilm was achieved with a power density of 16 mW m⁻².

42 43 Keywords: fungal microbial fuel cell; S. dehoogii; APAP/PAP/biomass; lignin; SEM/AFM

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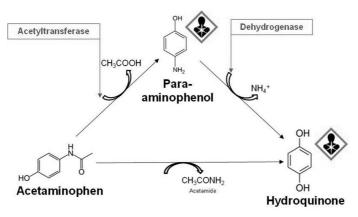
47 1. Introduction

48 The first recorded occurrence of electrochemical activity between bacterial/fungal (yeast) 49 species and electrodes can be traced back to the early 20th century. It was reported by Potter, 1911, 50 cultures of Escherichia coli and Saccharomyces spp. produced electricity using platinum 51 macroelectrodes in a battery type setup with sterile media. The story of electromicrobiology gained 52 new interest in 1963, when a NASA space program demonstrated the possibility of recycling and 53 converting human waste to electricity during space flights. In 1991, pioneering work by Habermann 54 and Pommer reported the long-term use of municipal wastewater by a microbial fuel cell (MFC) 55 without malfunction or maintenance. Moreover, this study reported indirect electron transfer (a 56 mechanism of electron transfer allowing specific bacteria to donate electrons) via soluble mediators. 57 In 1999, it was discovered that mediators were not essential components within MFC configurations, 58 thus allowing MFCs to be developed without the need for expensive mediators (see the review by 59 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 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).

66 Concerning filamentous fungi, the most intensively studied systems are yeast-based MFCs, 67 where direct electron transfer via cytochrome c was reported (Hubenova and Mitov, 2015). 68 However, recent findings indicate that other fungi also have redox-active enzymes capable of 69 electrogenic activity in MFC systems (Morath et al., 2012; Rahimnejad et al., 2015). Fungi belonging to 70 the group of white-rot (well-known pharmaceuticals (Marco-Urrea et al., 2009) and wood degraders) 71 were found to have an extracellular oxidative ligninolytic enzymatic system that enables them to 72 degrade lignin (Pollegioni et al., 2015). The enzymes of this system can also degrade various 73 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 Figure 1 for APAP.



81 82

83

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

46

84 Many polycyclic aromatic hydrocarbons (PAHs) are also CMR compounds that can be 85 degraded by several fungal enzymes (Kadri *et al.*, 2017).

86 Scedosporium dehoogii is a filamentous fungus belonging to a complex of five fungi strains 87 named Scedosporium apiospermum (Gilgado et al., 2008), living in soil (Alvarez and Sanhueza, 2016) 88 and able to use aliphatic and aromatic hydrocarbons as carbon and energy sources: it can therefore 89 be a good candidate for bioremediation purposes (Blasi *et al.*, 2016). This microorganism is mainly 90 found in polluted waters and contaminated soils (sewage sludge, stagnant water ...). Although there 91 are few data about the ecology of this filamentous fungus, it is known that its ecological 92 environment is strongly impacted by human activities and this is true for almost all fungi (Harms et 93 al., 2011). This was shown through its ability to metabolize hydrocarbons (especially aromatics) as a 94 source of carbon and energy (Blasi et al., 2016). This filamentous fungus is a model of bio-indicators 95 for anthropogenic pollution, and also a microorganism of choice for bioremediation, as recently 96 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/m² (Mbokou *et al.*, 2017).
 The power density obtained for variou
- 102

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 <i>et al.,</i> (2011))	
Saccharomyces cerevisiae	glucose	344	(Christwardana <i>et al.</i> (2017))	
Arxula adeninivorans	dextrose and glucose	28	(Haslett <i>et al.,</i> (2011))	
Candida melibiosica	various carbohydrates	60	(Hubenova and Mitov (2010))	
Candida sp.	glucose from wastewater	21	(Lee <i>et al.</i> , 2015)	
, Hansenula anomala	various carbohydrates	690 - 2900	(Prasad et al., 2007)	
Scedosporium dehoogii	APAP	6.5	(Mbokou <i>et al.,</i> 2017)	
Scedosporium dehoogii	APAP	50	This work	
Scedosporium dehoogii	Lignin	16	This work	

103

104 The present paper reports the results of an investigation into the potential bioremediation of 105 para-aminophenol (PAP) from wastewater and energy production in an MFC comprising an *S*. 106 *dehoogii* carbon felt anode. The oxidation of APAP and energy production were studied. The 107 feasibility of the biodegradability of PAP by *S. dehoogii* was also explored. Finally, the 108 biodegradability of several ligno-cellulosic materials (bagasse, rapeseed, cellulose, lignin) by *S.* 109 *dehoogii* was studied in Petri dishes and the natural lignin from sugarcane bagasse was tested for the 110 first time as fuel in a FMFC.

111 2. Experimental

112 2.1. Reagents

113 APAP was purchased from Sigma-Aldrich. A 0.1 M phosphate buffer solution (PBS) of pH 7.4 114 was used as supporting electrolyte. All other aqueous solutions were prepared from analytical grade 115 chemicals and deionized water with a pH of 6.5, conductivity < 1 ??S cm⁻¹ and TOC < 0.1 mg L⁻¹ (Elga 116 Lab Water ultrapure-water system, Purelab-UV-UF, Elga).

117 2.2. Apparatus and electrochemical measurements

118 All the electrochemical experiments were performed using a PG580 analyzer (Uniscan 119 Instruments, UK). The electrochemical software used was UiEchem version 3.27 (Uniscan 120 Instruments, Biologic company, France). A conventional three-electrode cell configuration was 121 employed. Working electrodes (0.071 cm² of geometric area) as recently published (Prasad et al., 122 2007) consisted in carbon paste electrodes (CPE) modified by cellulose fibers. The reference electrode 123 was a saturated calomel electrode (SCE). Platinum wire (10 cm length and 4 mm diameter) and grid 124 respectively used the counter (1 cm of diameter) were as electrodes for 125 APAP/PAP/4-hydroxybenzoate (4-HBz) analysis by CPE and biofilm polarization/cathode 126 modification. An aqueous sample was taken once daily from the anode compartment. The residual 127 concentration of APAP was electrochemically determined using square wave voltammetry (SWV) 128 on our homemade modified CPE. For APAP and PAP analyses, the SWV optimized parameters 129 were a frequency of 400 Hz, pulse height of 90 mV, scan increment of 15 mV and initial potential of 130 -0.2 V vs. SCE.

131 2.3. Strains and Materials

132 Four S. dehoogii strains UA 110350859-01, 110350889-01, 110354504-01 and 110350150-01, 133 isolated from soil samples in the vicinity of Angers (France) were used throughout this study in 134 order to find the best strain to use in the MFC device. The fungi were routinely maintained by 135 weekly passages on a yeast extract-peptone-dextrose (YPD) agar medium containing in g L⁻¹: yeast 136 extract, 5 g; peptone, 5 g; dextrose, 20 g and chloramphenicol, 0.5 g. The ability of Scedosporium 137 strains to use APAP, PAP, HQ and 4-hydroxybenzoate (4-HBz) as the sole carbon source was 138 investigated by cultivating the fungi on Scedo-Select III agar plates (Pham et al., 2015) containing 139 each molecule respectively, as follows. Conidia were harvested from 1-week-old cultures at 25-30°C 140 on YPD medium plates by flooding the agar surface with 15 mL of ultrapure water. The fungal 141 suspension was then filtrated on a 40 µm pore size sterile nylon filter and conidia were pelleted by 142 centrifugation at 4000 g (5 min at 4°C). They were resuspended in 10 mL of sterile ultrapure water, 143 and finally enumerated using a hemocytometer. Conidia were then inoculated onto Scedo-Select III 144 agar plates containing either APAP, 4-HBz, PAP or HQ, as carbon sources. For this purpose, stock 145 solutions of these pharmaceuticals (1 g each in 100 mL of ultrapure water) were prepared and 146 sterilized by filtration (0.2 µm pore size sterile membrane). After addition of the carbon source to the 147 culture medium at a final concentration of 0.9 g L⁻¹ and inoculation with conidia of S. dehoogii (10⁴ in 148 10 μ L), the plates were incubated during 1 week at 25°C. Growth of the fungus was followed by 149 measuring the diameter of the mycelium on the plate every day for 1 week (see Table 2 in the results 150 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 10⁶ spores mL⁻¹ was used for the formation of the biofilm on carbon felt.

158 2.4. Bioanode elaboration

159 Carbon Felt (CF) (Carbon Lorraine supplier, France) (CF geometrical area = 10 cm²) was used as
160 the substrate for fungal biofouling deposition. Prior to use, the CF was cleaned successively using a 1
161 M HCl solution and ultrapure water. Following the usual practice, the CF was then immersed in a
162 1:1 (volume ratio) mixture of ethanol-water for a few minutes followed by sonication 2 min. (47 kHz)
163 in ultrapure water to achieve ethanol rinsing. The CF was finally autoclaved at 120°C during 15 min.
164 before colonization by the fungus.

165 The CF anode was immersed in a suspension of *S. dehoogii*. The biofilm was then developed by 166 polarization of the CF at -0.15 V vs. SCE during one week under sterilized conditions (laminar oven) 167 (Figure S2, Supplementary Information). The sole source of carbon was 4-HBz at 0.9 g L⁻¹ for the 168 formation of the biofilm. As illustrated in Figure S2, above 90% of the 4-HBz was consumed after 7 169 days. The initial current density at the potential -0.15 V vs SCE was 175 mA m⁻² at t=0 and after 1 170 week attained the value of 375 mA m⁻². The potentiostat used was an electrochemical analyzer VASD 171 40, connected to a three-electrode system. Carbon felt, SCE and a platinum wire were the working 172 electrode, the reference electrode and the counter electrode, respectively. The homemade 173 cellulose-based CPE was used to follow the consumption of 4-HBz.

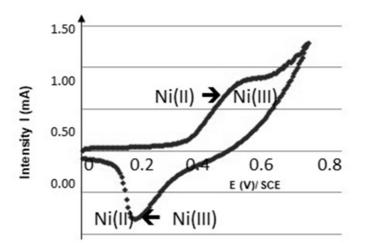
174 2.5. Preparation of CPE

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.

181 2.6. Cathode elaboration

182 The CF cathode was first electrochemically pretreated under the following conditions: CV 183 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 184 is essential to achieve good reproducibility of the poly-NiTSPc film deposition. The electrochemical 185 deposition of poly-NiTSPc was achieved in 0.1 M NaOH and 2 mM NiTSPc aqueous solution at a 186 fixed potential of +0.8 V vs. SCE during 3000 s in order to optimize the poly-NiTSPc matter to be 187 deposited. The effect of poly-NiTSPc electrodeposited on a carbon felt surface has been studied 188 previously (Champavert et al., 2015). Although the electrocatalytic effect of this film on oxygen 189 reduction has not been demonstrated, the presence of the poly-NiTSPc film was useful to reach a 190 larger surface area. In this study, preparation of the poly-NiTSPc film was optimized in terms of its

191 electrodeposition potential.



192

193

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

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 10⁴ 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 Figure 2.

204 2.7. Fungal Microbial biofuel cell set up

The FMFC set up was divided into two compartments, as recently reported (Mbokou *et al.*, 206 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.

211 The solution of the cathode chamber was also PBS. O_2 from air was provided to the cathode 212 chamber before filtration using a 0.45 μ m filter. An external resistance of 1 k Ω was connected to the 213 electrodes in order to shuttle electrons from anode to cathode. This external resistance was used to 214 determine the power output during MFC discharge. The MFC started to function when APAP (100 215 mg L-1) was added in the anode compartment. Residual APAP concentrations in this compartment 216 were measured daily using the SWV technique on the CPE as described above (see section 2.5). All 217 our experiments were carried out at room temperature. In open circuit the electromotive force was 218 measured at +550 mV. During the experiments measuring the power density we estimated that the 219 internal resistance of the MFC was around 5000 Ohm. Furthermore, as an initial control we did not 220 observe any power density in the absence of APAP (or lignin).

221

222 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.

228 In the case of Lignin as the carbon source in the FMFC, the phenolic composition of the medium was 229 analyzed before and after the experiment according to the Folin-Cioclateu method with 96-well 230 microplates. Each well was fed 20 µL of sample, then 10µL of commercial Folin-Ciocalteu reagent 231 and 170 µL of Na₂CO₃ alkaline solution at 2.36% were added. The optical density was read after 45 232 minutes of reaction at 700 nm with a specific plate reader (BMG-Labtech Spectrostar-Nano). This 233 colorimetric method was used with a calibration curve obtained with different concentrations of 234 gallic acid. The total concentration in phenolic compounds is therefore given in gallic acid 235 equivalent.

- 236 The phenolic monomer composition of the medium was characterized by HPLC on an OmniSpher 3
- 237 C18 100 x 4.6 column (Agilent Technologies). The gradient was as follows: 91% acidified water (1%
- 238 acetic acid (v/v)) and 9% acetonitrile for 25 min, from 9 to 90% acetonitrile in 5 min, kept constant for
- 239 5 min, then decreased back to 91% acidified water in 5 min; the column was equilibrated for 7 min
- 240 between runs. The flow rate was 0.5 mL/min, the injection volume was $10 \mu \text{L}$ and the column
- 241 temperature was maintained at 25 °C. The UV detector was set at 280 nm. Concentrations for the
- 242 calibration curves ranged between 0 and 200 mg/L. Standard and process samples were diluted in
- 243 acetonitrile: water at a ratio of 50:50 (v/v) prior to injection. Gallic acid, 4-hydroxybenzoic acid,
- 244 caffeic acid, syringic acid, vanillic acid (VA), 4-hydroxybenzaldehyde (4HBA), vanillin, *p*-coumaric

245 acid (*p*-CA), syringaldehyde, ferulic acid (FA), sinapic acid and hydroxycinnamic acid were 246 quantified.

- 247 2.9. Field emission gun scanning electron microscopy (FEGSEM) and atomic force microscopy (AFM)
- 248 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 FEGSEM observations.

255 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.

- 260 3. Results and discussion
- 261 3.1. Comparison of the growth of different S. dehoogii strains in the presence of different aromatic molecules

We compared the radial diameter growth (Figure 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).

265**Table 2.** Radial diameter (in mm) of the mycelium of 4 strains of *S. dehoogii* grown on266different carbon sources at 25°C.

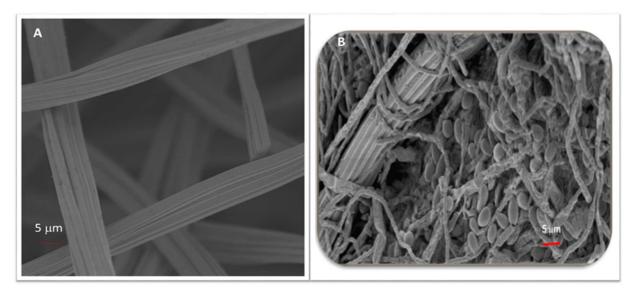
<u>Churcher</u> a	Source of carbon			
Strains	4-HBz	APAP	HQ	PAP
110350150-01 (1)	21	15	14	14
110354504-01 (2)	18	15	12	14
110350859-01 (3)	20	15	9	11
110350889-01 (4)	17	17	10	7

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.

270 3.2. Morphological and topographical characterizations of the fungal bioanode

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

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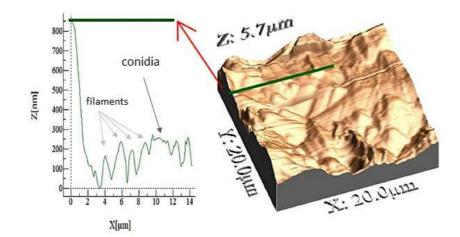


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Figure 3. 2D-FEG-SEM image of unmodified carbon felt (A) and carbon felt covered by a biofilm of Scedosporium dehoogii after polarization during 7 days (B).

278 After 1 week of polarization, conidia and filaments of the fungus became visible (center - right 279 of Figure 3). A CF fiber of 12 µm diameter was observed top left.

280 To complete FEGSEM observations, the topographical characterization of the biofilm was 281 drawn in 3D (Figure 4). A zoom on the green line in Figure 7 enabled measurement of the diameter 282 of the filaments (around 2 μ m) and that of conidia (around 5 μ m).



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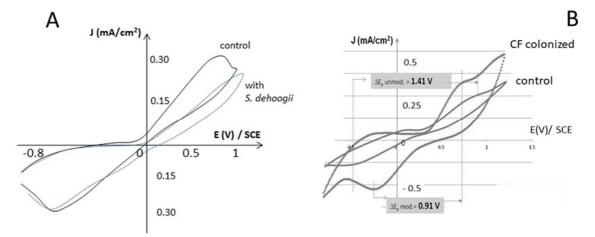
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Figure 4. AFM 3D-image of the S. dehoogii biofilm developed on the carbon felt.

285 Figures 3 and 4 are complementary illustrations of the presence of the S. dehoogii biofilm on the 286 CF anode (see also Figure S2B for a macroscopic view of the bioanode).

287 3.3. Proving the connection of S. dehoogii to CF

288 One of the technological barriers often encountered by bioelectrochemists is the connection of 289 the microorganisms to the electrodes, which does not necessarily operate directly. We studied the 290 kinetics of electron transfer for the redox system NAPQI/APAP (NAPQI, N-acetyl-p-benzoquinone 291 imine scheme 1) in order to compare the unmodified CF (control, Figure 5) and that covered by the 292 S. dehoogii biofilm (CF colonized by S. dehoogii).

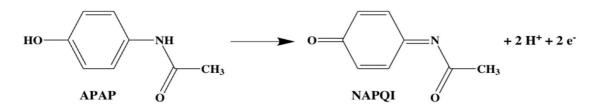


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Figure 5. Cyclic voltammograms of the carbon felt colonized or not, at a scanning rate of 200 mV s⁻¹, in the presence of APAP 50 mg L⁻¹ in PBS : **(A)** Non-colonized CF (control) without and with a suspension of *S. dehoogii*; **(B)** CF colonized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



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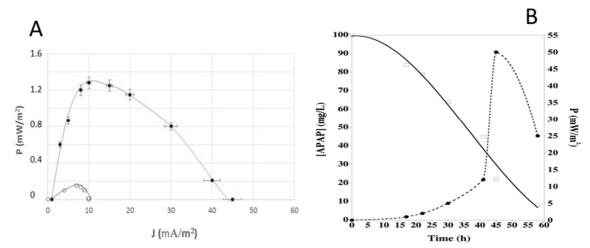
Scheme 1. Electrochemical oxidation of APAP.

Figure 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 Δ Ep 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 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.

308 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. Figure 6 shows the degradation kinetics of APAP along with the power density generated by the MFC.



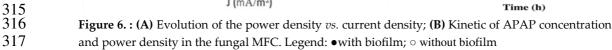


Figure 6A shows the polarization curves of the FMFC without and with biofilm. In both cases an optimal resistance was obtained and found to be around 3 000 Ohm at the maximum power density. In Figure 6B, the APAP concentration started to decrease along with the growth of the fungal biofilm on the anode. This means that APAP has been degraded by fungi and used as a carbon and energy source.

323

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).

332

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

336

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

 $C_8 H_9 O_2 N + \frac{37}{2} O_2 \rightarrow 8 CO_2 + \frac{9}{2} H_2 O + \frac{1}{2} N_2$

339

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

340 Then, in a the complete combustion of APAP 37 e- per mol. are delivered as in the case of glucose 341 only 24 e- are released.

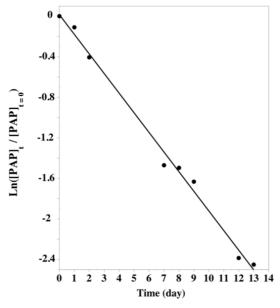
342

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.

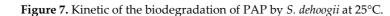
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350 3.5. PAP biodegradation studies

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



353 354

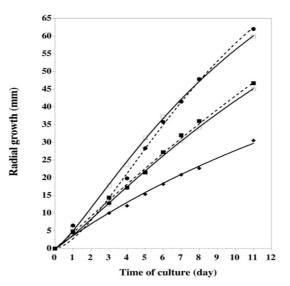


This confirmed the results reported in Table 2, showing that *S. dehoogii* is able to use PAP as a source of carbon in Erlenmeyer flasks. The kinetic follows a process of pseudo-order 1 with a kinetic constant of 0.19 day⁻¹, and a half-life of 3.3 days. This results are closer to those obtained by Mbokou (Mbokou *et al.*, 2017) for APAP biodegradation in the same FMFC set-up, where a kinetic constant of 0.11 day⁻¹ and half-life of 6.3 days were found.

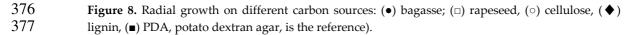
360 3.6. Plant biomass as the source of carbon for Scedosporium dehoogii

361 Wastewaters usually contain very low concentrations of pharmaceutical compounds (a 362 few µmol L-1 or less) and cannot allow our FMFC to attain a very high power density. Therefore, we 363 decided to test a cellulose-based source of carbon from ligno-cellulosic materials (LCM) as a new 364 source of fuel. LCM is mainly composed of cellulose, hemi-cellulose and lignin. They constitute the 365 most important source of organic carbon on earth and account for nearly all carbon sequestered 366 annually in plant matter (Mbokou et al., 2016b). Lignins are complex nonlinear polymers, whose 367 precursor is phenylalanine (Davin et al., 2008). In particular, lignins are a major source of aromatic 368 carbon on Earth, where it accounts for nearly 30% of carbon sequestered in plant biomass. There is a 369 great diversity of lignins. Plant polymers thus constitute very abundant energy sources whose 370 bioremediation reduces their carbon footprint. Numerous fungi are known to degrade LCM (for 371 example, Scedosporium apiospermum, Pleurotus ostreatus, Phanerochaete chrysosporium ...) because they 372 possess lignin-modifying enzymes (Morales et al., 2017).

Figure 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.



375



378 The results show for the first time that Scedosporium dehoogii can metabolize different plant 379 biomasses as a source of carbon and energy. As shown in figure 8, the rate of growth of the fungus 380 decreased following the order: bagasse - rapeseed > cellulose > lignin.

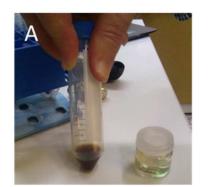
381 Based on these encouraging results, we explored the feasibility of lignin as biofuel in our FMFC.

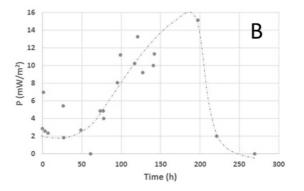
382 In fact, lignocellulosic or second biorefineries transform agricultural by-products or forest 383 biomass into energy (i.e. thermal), various chemicals and also materials (Oriez et al. 2019).

384 In the last part of our work, we therefore tested for the first time the biodegradability of 385 sugarcane bagasse lignin in our FMFC device.

386 3.7. FMFC performances with lignin

387 The fabricated FMFC started functioning when lignin 1 g.L-1 (first injection) was introduced into the 388 anode compartment.





389 390

391

Figure 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

392

393 Residual lignin species such as monolignols were not detected after 10 days residence time in the 394 anode compartment of the FMFC. A decolorization was observed as illustrated in Figure 9A. The 395 analysis of the medium with lignin showed that it had an initial concentration of 1.84 g GAE (Gallic

396 acid equivalent)/L. At the end of the experiment, the concentration was only 0.24 g GAE/L, meaning 397 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

399 of small molecules. It can be assumed that lignin was used as the source of carbon and completely

400 degraded by the strain, which can explain the sharp decrease in the electric production, after 200 h,

401 due to the lack of the carbon source.

402 The power density generated by the FMFC in the presence of lignin as a function of time was

 $403 \qquad \text{monitored, as illustrated in Figure 9B. It shows a maximum power density of 16 mW/m^2 for a time}$

- 404 of 200h.
- 405

406 4. Conclusions and perspectives

407 It has been demonstrated for the first time that a biofilm of *Scedosporium dehoogii* developed by 408 polarization at -0.15 V vs. SCE during one week established a connection to a CF bioanode. The 409 cathode of our fungal MFC presented an optimum potential of 0.8 V vs. SCE for $3.5\pm0.2 \mu$ m thickness 410 of poly-NiTSPc film. We have also shown that APAP is an efficient fuel for this FMFC. Stable output 411 performances were obtained giving a power density of 50 mW m² for an EMF of 550 mV.

412 Furthermore, the biodegradation of PAP, the main APAP by-product from bacterial 413 biodegradation was studied and *Scedosporium dehoogii* was able to use PAP as the sole source of

414 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

420 derivered by the fungal bioanode is considered better, a fact making *sceuosportum uenoc* 421 alternative for the development of novel FMFCs with sustainable carbon sources.

422

423 In the near future, we will conduct first experiments with other fungi as *Scedosporium dehoogii*

424 because very recent results demonstrated its previously suspected pathogenicity for human beings. 425 The goal will be to achieve a better characterization of the enzymes involved in the degradation

425 The goal will be to achieve a better characterization of the enzymes involved in the degradation 426 processes and to increase the power density developed by our FMFC.

427

428 To conclude we have often observed biofouling on the surface of the Nafion[®] membrane on the side 429 in contact with the fungal biofilm, antifouling strategies should be developed and tested and/or

in contact with the fungal biofilm, antifouling strategies should be developed and tested and/ordevelopment of a new proton exchange membranes under low cost and dedicated to its application

430 development of a new proton exchange memb 431 in microbial fuel cells.

432

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443 References

444	Alvarez, E., Sanhueza, C., 2016. New record of Scedosporium dehoogii from Chile: Phylogeny and susceptibility
445	profiles to classic and novel putative antifungal agents. Rev. Iberoam. Micol. 33, 224-229.
446	
447	ATSDR, 2017. Agency for toxic substances and disease registry, U.S. Dep. Health Hum. Serv. USA.
448	http://www.atsdr.cdc.gov/SPL/index.html
449	
450	Bennetto, H.P., Stirling, J.L., Tanaka, K., Vega, C.A., 1983. Anodic reactions in microbial fuel cells. Biotechnol.
451	Bioeng. 25, 559–568. DOI:10.1002/bit.260250219
452	
453	Bergel, A., Féron, D., 2002. French Patent CNRS 6 August 2002, (FR 02/100009).
454	
455	Blasi, B., Polynter, C., Rudavsky, T., 2016. Pathogenic yet environmentally friendly? Black fungal candidates for
456	bioremediation of pollutants. <i>Geomicrobiol J.</i> 33, 308-17 and references therein.
457	https://www.tandfonline.com/doi/full/10.1080/01490451.2015.1052118
458	
459	Champavert, J., Ben Rejeb, S., Innocent, C., Pontié, M., 2015. Microbial fuel cell based on Ni-tetra sulfonated
460	phthalocyanine cathode and graphene modified bioanode. J. Electroanal. Chem. 757, 270-276.
461	http://dx.doi.org/10.1016/j.jelechem.2015.09.012
462	
463	Christwardana, M., Kwon, Y., 2017. Yeast and carbon nanotube based biocatalyst developed by synergetic
464	effects of covalent bonding and hydrophobic interaction for performance enhancement of membraneless
465	microbial fuel cell. Bioresour. Technol. 225, 175–182. DOI: 10.1016/j.biortech.2016.11.051
466	
467	Davin, L.B., Jourdes, M., Patten, A.M., Kim, K.W., Vassao, D.G., Lewis, N.G., 2008. Dissection of lignin
468	macromolecular configuration and assembly: comparison to related biochemical processes in allyl/propenyl
469	phenol and lignan biosynthesis. Nat. Prod. Rep. 25, 1015-1090.
470	
471	de Dios M.A.F., del Campo A.G., Fernandez, , F.J., Rodrigo, M., Pazos, M. , Sanroman M.A., 2013. Bacterial-
472	fungal interactions enhance power generation in microbial fuel cells and drive dye decolorization by an ex situ
473	and in situ electro-Fenton process. <i>Bioresource Technology</i> . 148, 39-46.
474	
475	Gilgado, F., Cano, J., Gené, J., Sutton, D.A., Guarro, J., 2008. Molecular and phenotypic data supporting distinct
476	species statuses for Scedosporium apiospermum and Pseudallescheria boydii and the proposed new species
477	Scedosporium dehoogii. J Clin Microbiol. 46, 766–771.
478	
479	Habermann, W. and Pommer E –H, 1991, Biological fuel cell with sulphide storage capacity, <i>Appl.</i>
480	Microbial Biotechnol. 35, 128-133
481 482	Harma H. Schlosson D. Wield I. V. 2011 Hatamad actantich and still for the history disting (1)
482 483	Harms, H., Schlosser, D., Wick, L.Y., 2011. Untapped potential: exploiting fungi in bioremediation of hazardous
485 484	chemicals. Nat. Rev. Microbiol. 9, 177–192.
+0+	

485	Haslett, N.D., Rawson, F.J., Barriere, F., Kunze, G., Pasco, N., Gooneratne, R., Baronian, K.H.R., 2011.
486	Characterization of yeast microbial fuel cell with the yeast Arxula adeninivorans as the biocatalyst. Biosens.
487	Bioelectron. 26, 3742–3747. DOI:10.1016/j.bios.2011.02.011.
488	
489	Hubenova, Y., Mitov, M., 2015. Extracellular Electron transfer in yeast-based biofuel cells: A review.
490	Bioelectrochemistry. 106, 177-185. doi.org/10.1016/j.bioelechem.2015.04.001
491	
492	Hubenova, Y., Mitov, M., 2010. Potential application of Candida melibiosica in biofuel cells. Bioelectrochemistry.
493	78, 56–61. DOI:10.1016/j.bioelechem.2009.07.005
494	
495	Kadri, T., Rouissi, T., Kaur Brar, S., Cledon, M., Sarma, S., Verma, M., 2017. Biodegradation of polycyclic
496	aromatic hydrocarbons (PAHs) by fungal enzymes: A review. J. Environ. Sci. 51, 52–74.
497	
498	Langenhoff, A., Inderfurth, N., Veuskens, T., Schraa, G., Blokland, M., Kujawa-Roeleveld, K., Rijnaarts, H., 2013.
499	Microbial removal of the pharmaceutical compounds Ibuprofen and diclofenac from wastewater. Biomed. Res.
500	Int., 2013, 325806
501	
502	Lee, YY., Kim, T.G., Cho, KS., 2015. Isolation and characterization of a novel electricity-producing yeast,
503	Candida sp. IR11. Bioresour. Technol. 192, 556–563. DOI: 10.1016/j.biortech.2015.06.038
504	
505	Marco-Urrea, E., Pérez-Trujillo, M., Vicent, T., Caminal, G., 2009. Ability of white-rot fungi to remove selected
506	pharmaceuticals and identification of degradation products of ibuprofen by Trametes versicolor. Chemosphere.
507	74, 765–772.
508	
509	Mashkour, M., Rahimnejad, M., Mashkour, M., 2016. Bacterial cellulose-polyaniline nano-biocomposite: A
510	porous media hydrogel bioanode enhancing the performance of microbial fuel cell. Journal of Power Sources. 325,
511	322-328.
512	
513	Mbokou, F.S., Kenfack Tonle, I., Pontié, M., 2017. Development of a novel hybrid biofuel cell type APAP/O2
514	based on a fungal bioanode with a Scedosporium dehoogii biofilm. J. Applied Electrochemistry. 47, 273-280.
515	doi.org/10.1007/s10800-016-1030-5
516	
517	Mbokou, F.S., Pontié, M., Razafimandimby, B., Bouchara, JP., Njanja, E., Tonle Kenfack, I., 2016a. Evaluation
518	of the degradation of acetaminophen by the filamentous fungus Scedosporium dehoogii using carbon-based
519	modified electrodes. Anal. Bioanal. Chem. 408, 5895-5903. DOI 10.1007/s00216-016-9704-8.
520	
521	Mbokou, FS, Pontié M, Bouchara J-P, Tchieno MMF, Njanja E, Mogni A, Pontalier YP, Tonle KI 2016 ^b .
522	Electroanalytical Performance of a Carbon Paste Electrode Modified by Coffee Husks for the Quantification of
523	Acetaminophen in Quality Control of Commercialized Pharmaceutical Tablets. Int J Electrochem Volume 2016,
524	Article ID 1953278, http://dx.doi.org/10.1155/2016/1953278
525	
526	

527	Morales, L.T., Gonzalez-Garcia, L.N., Orozco, M.C., Restrepo, S., Vives, M.J., 2017. The genomic study of an
528	environmental isolate of Scedosporium apiospermum shows its metabolic potential to degrade hydrocarbons.
529	Stand. Genomic Sci. 12, 71.
530	
531	Morath, U. S, Hung R., Bennett W. J. 2012. Fungal volatile organic compounds: A review with emphasis on their
532	biotechnological potential. Fungal Biology Reviews. 26, 73-83.
533	
534	Rahimnejad M., Adhami, A. Darvari, S. Zirepour, A., Oh, S.E. 2015. Microbial fuel cell as new technology for
535	bioelectricity generation: A review. Alexandria Engineering Journal. 54, 745-756.
536	
537	Ghasemi, M., Wan Daud, W.R., Ismail, M., Rahimnejad M., Fauzi A., Leong, X., Miskan, M., Ben Liew, K 2013.
538	Effect of pre-treatment and biofouling of proton exchange membrane on microbial fuel cell performance.
539	International Journal of Hydrogen Energy. 38, 5480-5484.
540	
541	Rahimnejad, M., Ghasemi, M., Najafpour D.G., Ismail, M., Mohammad, W.A., Ghoreyshi, A.A. Sedky H.A
542	2016. Synthesis, characterization and application studies of self-made Fe3O4/PES nanocomposite membranes in
543	microbial fuel cell. Electrochimica Acta. 85, 700-706.
544	
545	Oriez.V., Peydecastaing.J., Pontalier.P.I. 2019. Separation of sugarcane bagasse mild alkaline extract
546	components by ultrafiltration-membrane screening and effect of filtration parameters. Process Biochemistry. 78,
547	91-99. Doi: 10.1016/j.procbio.2019.01.006.
548	
549	Pham, T., Giraud, S., Schuliar, G., Rougeron, A., Bouchara, JP., 2015. Scedo-Select III: a new semi-selective
550	culture medium for detection of the Scedosporium apiospermum species complex. Med. Mycol. 53, 512-519.
551	
552	Potter: M.C, 1911 Electrical effects accompanying the decomposition of organic compounds, Proc. R
553	Soc London, B 84, 260-276
554	
555	Prasad, D., Arun, S., Murugesan, M., Padmanaban, S., Satyanarayanan, R.S., Berchmans, S., Yegnaraman, V.,
556	2007. Direct electron transfer with yeast cells and construction of a mediatorless microbial fuel cell. Biosens.
557	Bioelectron. 22, 2604–2610. DOI:10.1016/j.bios.2006.10.028
558	
559	Pollegioni, L., Tonin, F., Rosini, E., 2015. Lignin-degrading enzymes. FEBS J. 282, 1190–213.
560	
561	Pontie, M., Mbokou, S., Bouchara, JP., Razafimandimby, B.J., Pontalier, P.Y., 2017. Paracetamol sensitive
562	cellulose-based electrochemical sensors. J. Renewable materials. 6, 242-250. doi.org/10.7569/JRM.2017.634169
563	
564	Rodarte-Morales, A.I., Feijoo, G., Moreira, M.T., Lema, J.M., 2012. Biotransformation of three pharmaceutical
565	active compounds by the fungus Phanerochaete chrysosporium in a fed batch stirred reactor under air and oxygen
566	supply. Biodegradation. 23, 145–156.
567	

- 568 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. *Bioresource technology*,
 102(3), 2751-2757.
- 571 Sekrecka-Belniak, A., Toczyłowska-Maminska, R., 2018. Fungi-Based Microbial fuel cells. *Energies*. 11,
 572 2827-2845. doi:10.3390/en11102827.
- 573

575

574 Slate, A.J., Whitehead, K.A., Brownson, D.A.C., Banks, C.E., 2019. Microbial fuel cells: An overview of current

and

references

therein.

576 577

doi.org/10.1016/j.rser.2018.09.044.

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

technology. Renewable and Sustainable Energy Reviews. 101, 60-81

- 580
- 581