LOW COST PAPER/WO_3 NANOCOMPOSITE FOR COLORIMETRIC DETECTION OF ELECTROCHEMICALLY ACTIVE BACTERIA

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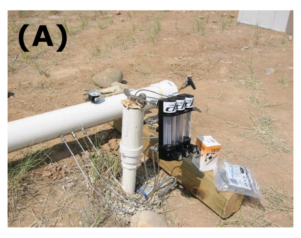
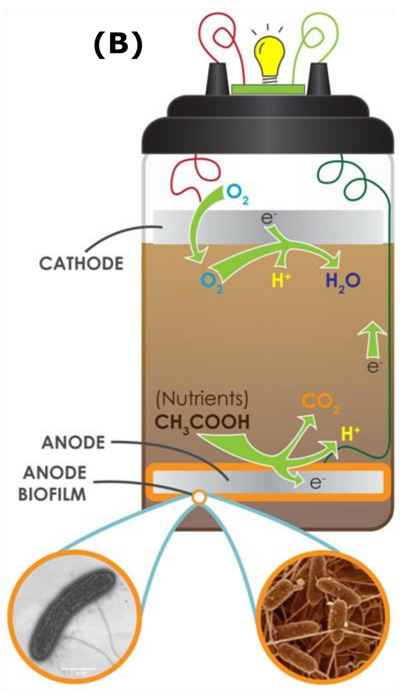
INTRODUCTION

Electrochemically active bacteria (EAB) are able to transfer electrons to extracellular electron acceptors [1] which provides singular characteristics for the development of biotechnological applications in bioremediation (A) and microbial electricity production (B) [1-2]. Although an increasing variety of EAB have been isolated, so far the number of isolated species is still very limited which has substantially constrained fundamental understanding about their roles in environment. This work reports a colorimetric sensor which detects the presence of the anaerobic bacteria *Geobacter sulfurreducens.* The experimental procedure was based on lab-on-paper technology [3] and provides a rapid response (~4h) compared to the conventional methods (~5 to 6 days).

(A)



(B)

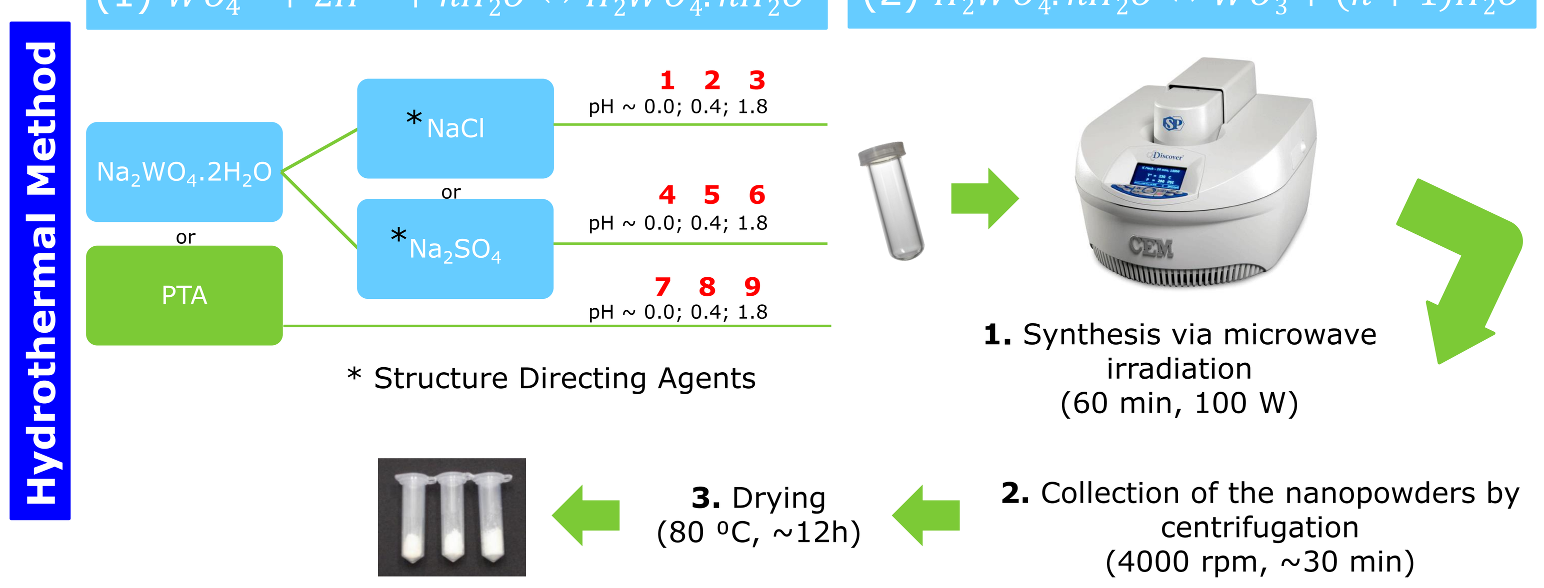


EXPERIMENTAL METHODS

Tungsten Oxide Synthesis

(1) $WO_4^{2-} + 2H^+ + nH_2O \leftrightarrow H_2WO_4.nH_2O$

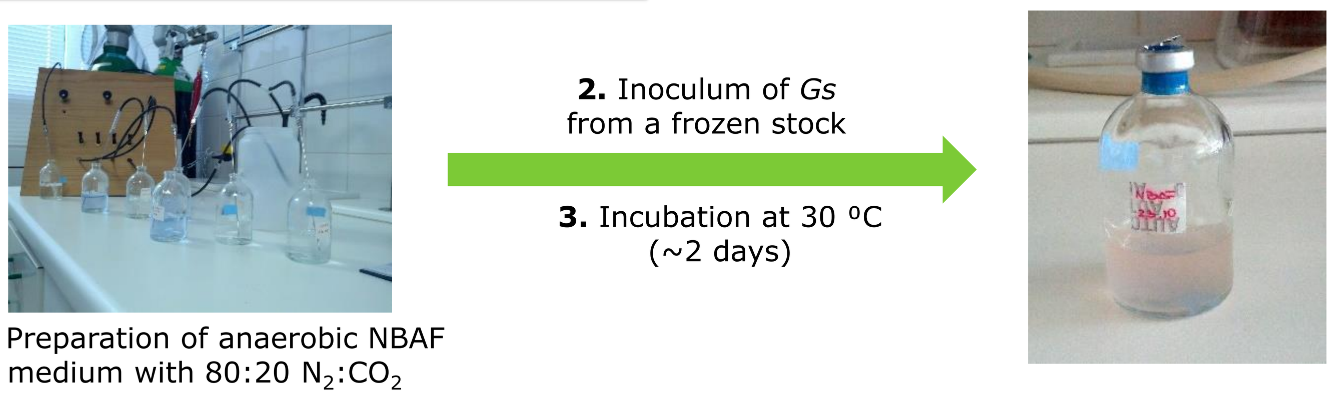
(2) $H_2WO_4.nH_2O \leftrightarrow WO_3 + (n+1)H_2O$



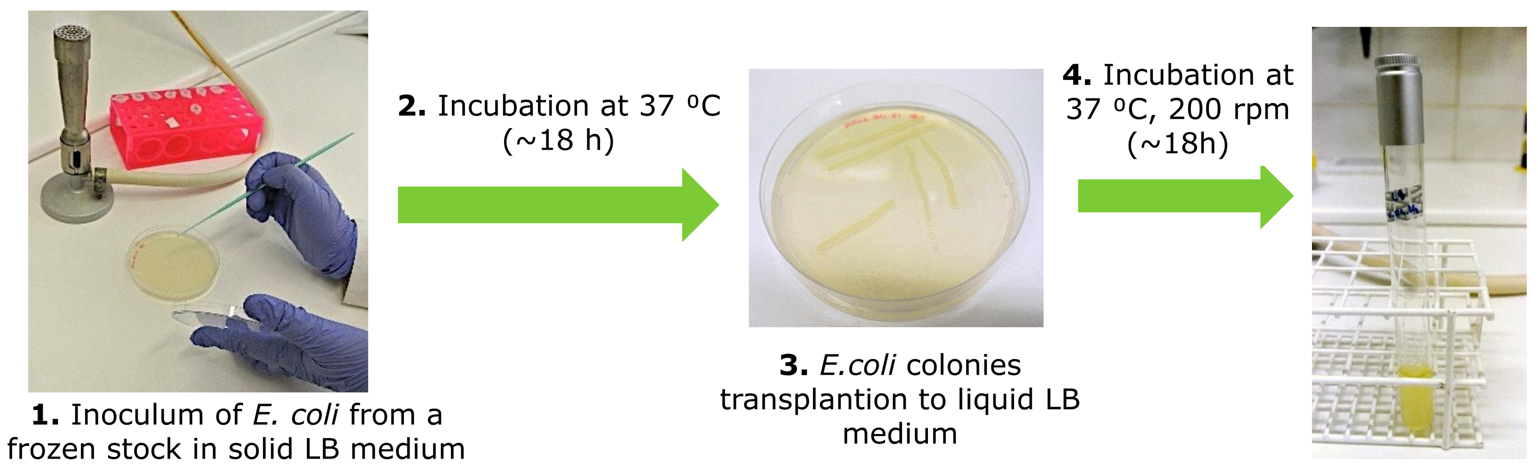
Paper Sensor Fabrication

Bacterial Growth

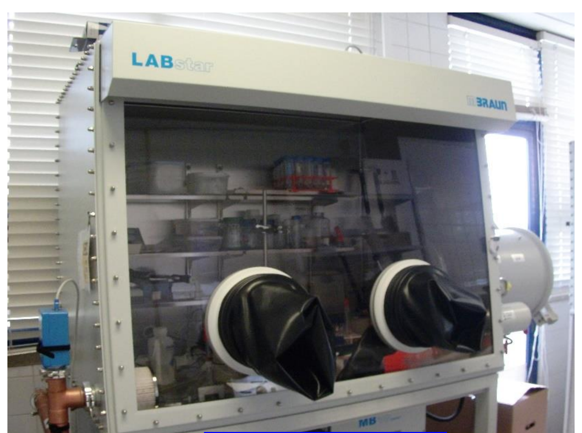
Growth of EAB *Geobacter sulfurreducens* (Gs)



Growth of *Escherichia coli* (*E. coli*) – negative control



Colorimetric Detection of EAB



Anaerobic chamber

1. *Gs* and *E. coli* cells collection by centrifugation (4000 rpm, 5 min);

2. Resuspention of *Gs* and *E. coli* in a buffer solution;

3. Addition of 50 μL of *Gs* to the sample well and *E. coli* to the control well;

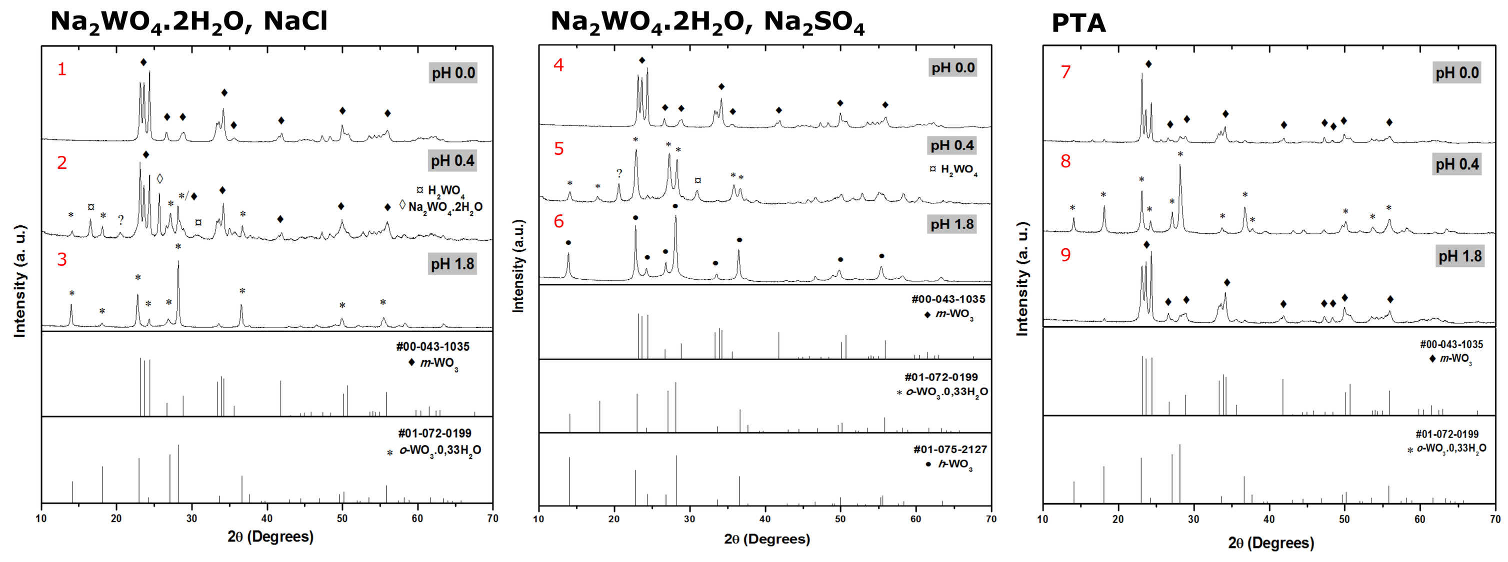
4. Drying;

5. Appearance of final results (~4h): positive result (blue) or negative result (white);

RESULTS

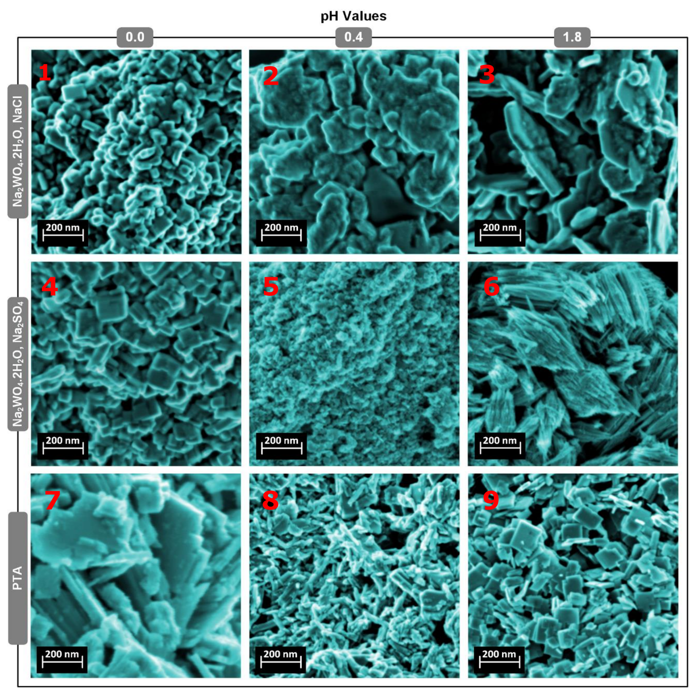
Morphological, Structural and Optical Characterization of Tungsten Oxide Nanoparticles

X-rays Diffraction



For pH~0 the structure is always monoclinic, independently of the precursors and/or the structure directing agents.

Scanning Electron Microscopy



UV-Visible Spectroscopy

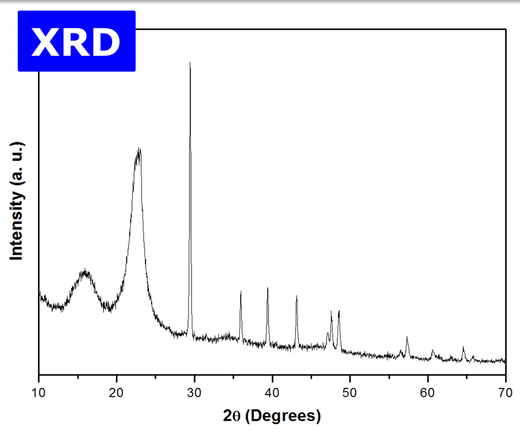
Precursor	Structure Directing Agent	Geometry	Optical Hiatus [eV]
$Na_2WO_4.2H_2O$	NaCl	Monoclinic	2.53
		Orthorombic + Monoclinic	3.13
		Orthorombic	3.64
	Na_2SO_4	Monoclinic	2.34
		Orthorombic	3.17
		Hexagonal	4.03
PTA	-	Monoclinic	3.97
		Orthorombic	3.89
		Monoclinic	3.89

$Na_2WO_4.2H_2O$: monoclinic < orthorombic < hexagonal
PTA: monoclinic = orthorombic

Substrate Characterization

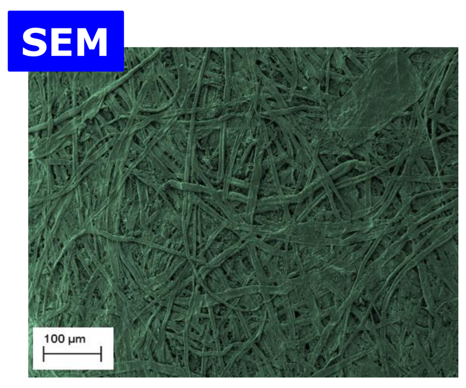
Portucel – Papel de Escritório Português 300%

XRD



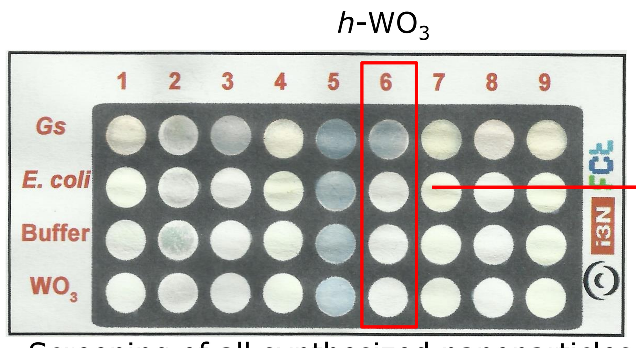
Characteristic peaks of type I cellulose.

SEM

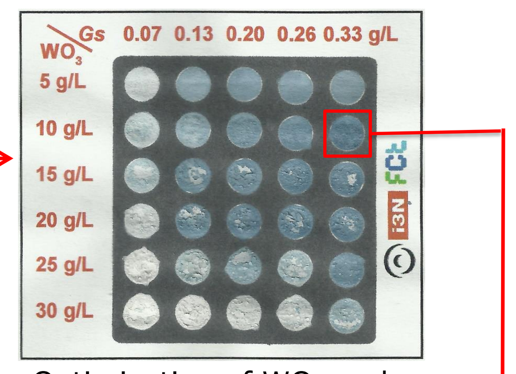


EAB Sensor

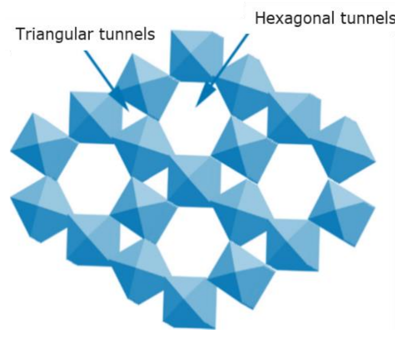
h-WO_3



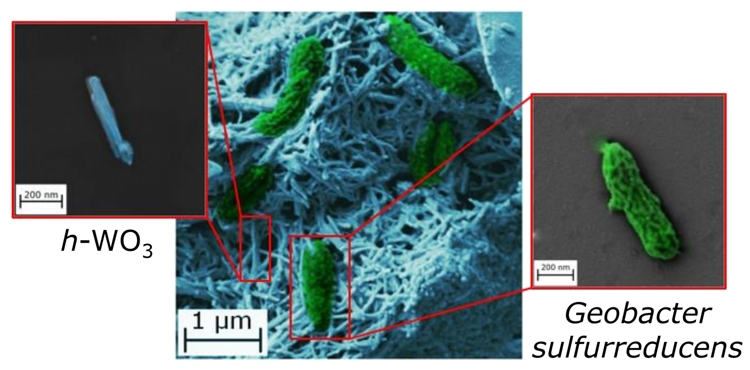
Screening of all synthesized nanoparticles (WO_3 5g/L and Gs 0,33 g/L).



Optimization of WO_3 and *Gs* concentration.



Crystallographic structure of *h*-WO_3



$WO_3\,(white) + xe^- + xH^+ \leftrightarrow HxWO_3\,(blue)$

CONCLUSIONS

- WO_3 nanoparticles were successfully produced by hydrothermal method via microwave irradiation;
- The influence of the precursor, structure directing agent and pH were studied and allowed the production of different crystallographic and morphologic WO_3 structures;
- The distortion in the crystal lattice in the orthorhombic and hexagonal geometries leads to lowering of the valence band and raising the conduction band with a consequent increase in energy gap;
- The enhanced electrochromic properties of the hexagonal WO_3 can be attributed to its tunnel structure that enables the inserting of protons and, consequently, the formation of $HxWO_3$;
- For the EAB sensor, both WO_3 (10 g/L) and *Gs* (0.33 g/L) concentration were optimized for the best adhesion and color contrast;
- The produced sensor provides an innovative, rapid, effective, inexpensive and high throughput test for identification of EAB and evaluation of their extracellular electron transfer capabilities.

References:
[1] D. R. Lovley, *et al*, *Adv. Microb. Phys.*, 59, 0065-2911 (2011).
[2] S. J. Yuan, *et al*, *Scient. Rep.*, 3, 1315 (2013).
[3] M. N. Costa, *et al*, *Nanotechnology*, 25, 094006 (2014).

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