Int. J. Electrochem. Sci., 10 **(2015)** 8886 - 8898

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Electropolymerized Neutral Red as Redox Mediator for Yeast Fuel Cell

Ummy Mardiana[1,2,3], *Christophe Innocent*[1*], *Haytem Jarrar*[1], *Marc Cretin*[1], *Buchari*[2], *Suryo Gandasasmita*[2]

[1] Institut Europeen des Membranes, ENSCM-UM2-CNRS, (UMR 5635), Universite de Montpellier, CC047, place E. Bataillon, 34095 Montpellier Cedex 5, France
[2] Institut Teknologi Bandung, Jalan Ganesha 10 Bandung, West of Java Indonesia 40132
[3] STIKes Bakti Tunas Husada Tasikmalaya, Jalan Cilolohan 36 Tasikmalaya, West of Java Indonesia 46115
[*]E-mail: christophe.innocent@univ-montp2.fr

Received: 24 March 2015 / *Accepted:* 3 September 2015 / *Published:* 30 September 2015

The electropolymerization of neutral red (NR) led to the formation of a redox active layer on Carbon Felt (CF) to mediate glucose oxidation at the anode of a microbial fuel cell (MFC) based on the use of *Saccharomyces cerevisiae*. The electropolymerization was conducted by cyclic voltammetry at pH 6 in phosphate buffer with or without yeasts in solution to prepare poly-neutral red (PNR) films entrapping yeasts or raw PNR films respectively. Both types of modified electrodes were electrochemically characterized showing that PNR is an efficient redox film for glucose biocatalytic oxidation using *Saccharomyces cerevisiae*.

Keywords: Poly-Neutral Red (PNR), modified electrode, *Saccharomyces cerevisiae*

NOMENCLATURE

Symbol	
CF	= Carbon Felt
NR	= Neutral Red
PNR	= Poly-Neutral Red
CF(Y)	= CF tested in a solution containing yeast (Y)
CF(Y-NR)	= CF tested in a solution containing yeast (Y) and NR
CF/PNR	= PNR polymerized on CF
CF/PNR-Y	= Yeast entrapped within PNR polymerized on CF

1. INTRODUCTION

To face an unavoidable lack of affordable energy in a near future and the still increasing pollution generated by the greenhouse gas emissions, it appears primordial to develop new and sustainable alternatives to common energy resources. One critical, renewable and primary energy source is directly generated upon the conversion of sustainable biomass. The last years, research has been focusing on the microbial fuel cells (MFCs) to address the development of new energy sources from biomass [1-3]. In the literature, two categories of MFCs are found depending on the way the electrons generated by the bacteria are transferred to the anode. Therefore, energy is produced either directly by using a mediator placed between the bacteria and the anode or without, electrons being driven from the electrochemical active sites of the bacteria to the anode.

Concerning the direct mode, the electrons transfer occurs from the outer membrane of the proteins such as the cytochromes [4, 5], nanowires [6] or trans-membranar proteins [7]. Commonly, micro-organisms such as *Shewanella putrefaciens*, *Geobacter sulfureduces* allow direct electrons transfer in fuel cells without adding exogenous mediators [4, 5]. In previous studies, yeasts have then been applied as biocatalysts in microbial fuel cells [8-11] and mediator-less MFCs have also been investigated [7, 12, 13]. In mediator-less MFCs, Sayed et al. [12] identified direct electrons transfer from the yeast to the anode. However, the current problem of fuel cell based on yeast (*i.e Saccharomyces cerevisiae)* is that energy produced from yeast fuel cell remains too low, mainly due to the very low rate of information transfer between living cell and anode surface.

Focusing now on the indirect mode, the electrons transfer occurs from the bioagent to the anode or the cathode via a chemical used as mediator generally artificial redox molecules. The presence of a mediator appears to improve drastically the transfer of electrons to the electrode [9, 14, 15]. Potter et al. [1] showed that *Saccharomyces cerevisiae* and *E.coli*, generated an anode potential in presence of a redox mediator and that the cell could not produce electricity without this redox molecule.

Thus, there are many concerns for studying and improving electrons transfer in yeast-based microbial fuel cells. Therefore, new methods have to be explored to enhance the mechanism of electrons transfer from a yeast cell towards the extracellular solid electrode. It is well known that rapid electrons transfer from a biocatalyst to the anode might be realized by applying appropriate electron mediator directly in the culture solution. Ideally, a redox mediator should have a good electrochemical reversibility as well as a redox potential close to the redox potential of the biocatalyst. Park *et al* [16] have shown that NR is a proper redox molecule showing the latter properties to be used in MFCs. However, using a mediator dissolved in the culture solution may represent a disadvantage since it leads to high operation cost and possible contamination of the MFC environment [7, 12]. To overcome this drawback, the mediator can be physically adsorbed onto the electrode; however, this also led to a low stability of the MFCs due to a rapid loss of the mediator from the anode surface upon cycling [17]. Finally, from a sustainable green energy power production point of view, it becomes essential to develop systems working with electrons carriers strongly immobilized at the electrode.

With these perspectives in mind, we have been working on the immobilization of the NR used as redox mediator molecule directly at the electrode by electropolymerization process. Indeed, the

electropolymerization of NR will form a NR polymeric film acting thus as a network including many electrons carriers that will further prevent the mediator from leaching upon MFCs utilization cycles [18]. Redox polymers such as poly-methylene blue [18, 19], poly-methylene green [20], poly-toluidine blue O [21, 22] have been reported. However NR appears to be the most promising monomer in term of electron transfer properties [9, 16, 23-26].

The use of PNR as redox polymer has thus been intensively studied in electroanalysis in order to improve performances of electrodes, through a better electrons transfer in biosensors [27-31]. Different supports were also envisaged to optimize the electropolymerization conditions of NR such as Indium Tin Oxide (ITO) conductive glass [32], glassy carbon [31, 33, 34] and platinum electrodes [33, 35]. Besides electropolymerization, other immobilization techniques have been explored to produce powerful mediator through electrons transfer enhancement such as direct covalent attachment to the electrode [16, 36] or direct chemisorption of a polymer bearing redox functional groups mixed to a carbon paste [37]. In the present paper, we have been demonstated Saccharomyces cerevisiae yeast as biocatalyst and studied the conditions of electrogeneration of a polymer film of NR at the surface of the anode of a yeast-based MFC. We are also comparing results obtained from the anodic half-cell of the MFC that is composed of *Saccharomyces cerevisiae* as biocatalyst both working directly in solution and entrapped within the PNR film. The development of the PNR film aims in this context to improve the stability of the anodic part of the MFC.

2. EXPERIMENTAL METHOD

All chemical used in this work were of analytical grade.

2.1. Substrate conditioning

Carbon Felt (CF) (Alfa Caesar, 5 cm x 1.5 cm and 1.27 cm thick) was used as substrate for NR electropolymerization. Prior use, CF was cleaned successively using a 1M HCl solution and ultrapure water. CF was then immersed in a 1:1 (volume ratio) mixture of ethanol-water for a few minutes followed by sonication in ultrapure water [23]. Finally, the surface of the CF was electrochemically activated by applying a potential cycling between -0.5 V and 1.5 V/SCE at a scan rate of 50 mV/s in a 1M H_2SO_4 solution until a stable voltammogramm was obtained.

2.2. Procedure of electropolymerization

The electropolymerization of NR (Sigma) was conducted by cyclic voltammetry using a potentiostat VERSASTAT 3 (PAR AMETEK). A 1 mM NR aqueous solution was prepared in presence of a 0.025 M phosphate buffer (PB) at pH 6 and a 0.1 M KNO_3 electrolyte solution (Sigma Aldrich). The PB at pH 6 was prepared from appropriate amounts of sodium dihydrogenophosphate ($NaH_2PO_4.2H_2O$) and disodium hydrogen phosphate (Na_2HPO_4) both purchased from Sigma Aldrich.

The sweeping potential range (scan rate of 50 mV/s) was set from -1.0 to 0.6 V/SCE. Platinum and KCl Saturated Calomel Electrode (SCE) were chosen as Counter Electrode (CE) and Reference Electrodes (RE), respectively.

2.3. Characterization of the PNR film

The electrochemical characterization of the generated PNR film was performed by chronoamperometry. The potential applied to the electrochemical half-cell was 0.3 V/SCE. The half-cell consisted in a 100 mL of a test solution, CF as working electrode (WE), SCE as RE and platinum as CE. A control experiment without any yeast showed a baseline current of 5 $\mu A.m^{-2}$ at the anode of the electrochemical half-cell upon polarization at 0.3V/SCE. The experiments were repeated three times and average values were given with experimental deviation.

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray spectroscopy (EDX) were performed on a Hitachi S-4800 and a Hitachi S-4500, respectively. The procedure to prepare samples was the following: a piece of the modified felt was carefully cut to a 1 cm x 1 cm dimension and dipped for 4h in an aqueous solution containing 4% of glutaraldehyde to stabilize the microorganisms. After rinsing the samples twice in the phosphate buffer solution, they were dehydrated in ethanol series 40, 60, 80, 100 vol. % for 30 min. before drying in a desiccator for 3h.

2.4. Preparation of the MFC

The total MFC was built in a dual compartment configuration with anodic and cathodic compartments. The two compartments (of 100 mL each) composing the dual chamber were separated by a Nafion® 117 (DuPont, USA) as proton exchange membrane. CF modified by PNR (CF/PNR) (5 cm x 1.5 cm x 1.27 cm) and nickel plate (5 cm x 1.5 cm) were used as anode and cathode electrodes, respectively. A scheme of the MFC system is shown in Fig.1. Each chamber was hermetically closed when operating to avoid loss of solutions through evaporation.

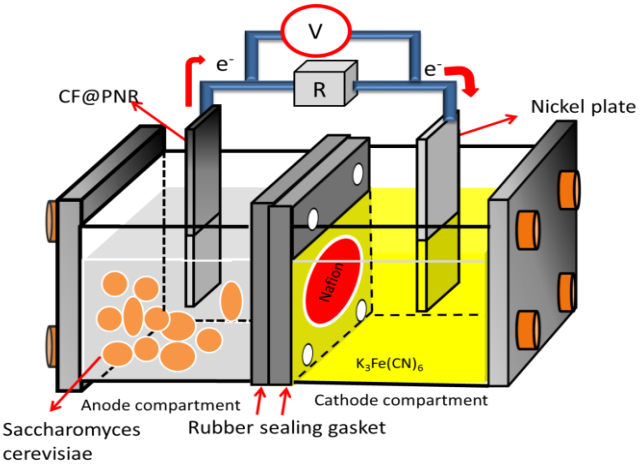


Figure 1. Scheme of the Microbial Fuel Cell (MFC) system

The anode compartment was filled with a solution of glucose monohydrate at 0.1 M in 0.1M PB pH 6 containing 2 g/100 mL of the yeast *Saccharomyces cerevisiae*as. The cathode compartment was filled with a solution of potassium ferricyanide (PF) (Sigma Aldrich) at 0.02 M in PB pH 6 as described in a previous study [14]. The electrons generated by the glucose oxidation at the anode promoted the reduction of potassium ferricyanide to ferrocyanide at the nickel plate. Oxygen presents in the cathodic chamber reoxidized ferrocyanide (Fe (II)) to ferricyanide (Fe (III)) allowing thus the MFC functioning. The voltage (E (V)) generated was recorded using a digital multimeter Voltcraft (model VC 850) and the current (I (A)) was calculated from the equation I = E/R with R (Ω) being the resistance of the system. Various resistances (from 100 Ω to 3 kΩ) were used as external loads to determine the maximal power output as previously presented [14]. MFC process was operated at 25 ± 1^{o}C.

3. RESULTS AND DISCUSSION

3.1 Electropolymerization and characterization of CF/PNR

The cyclic voltammogram of NR leading to the electropolymerization growth of PNR film after 15 scans and SEM image of the CF/PNR membrane are shown in Fig.2.

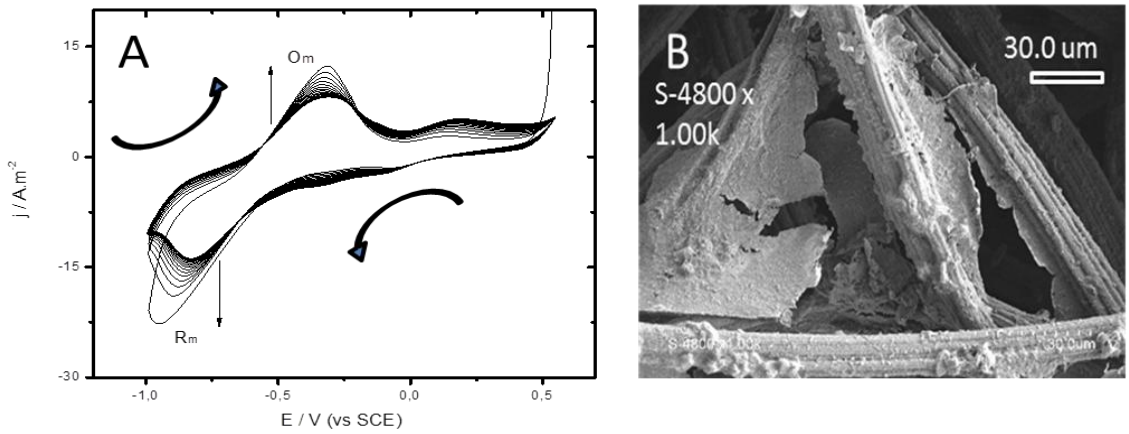
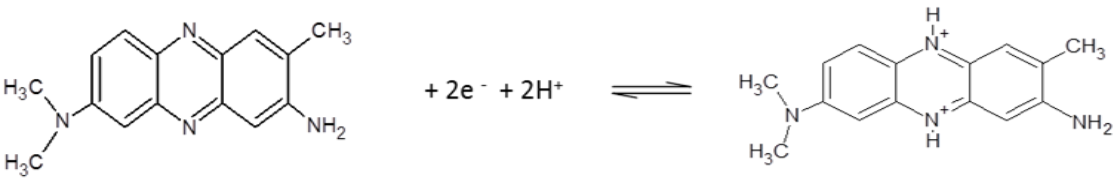


Figure 2. (A) Cyclic voltammogram of PNR (15 scans) and (B) SEM image of the CF/PNR membrane.

At high anodic potentials (> 0.5V/SCE), an irreversible oxidation of the monomer occurs. Upon cycling from -1V to 0.6V/SCE, the current peaks related to the redox activity of NR increase until 15 cycles indicating film growth. Previous experiments (not presented here) have shown that after 20 and 30 cycles, the anodic current peak is decreasing when for 15 cycles the anodic current peak reaches 12 $A.m^{-2}$. For an electrocatalytic application, the optimal value is then 15 cycles to attain the

higher redox activity of the PNR film. Considering Fig. 2 (A), O*m* and R*m* are related to oxidation and reduction potentials of NR, corresponding to the following equilibrium [38]:



(1)

After electropolymerization, oxidation and reduction peaks are located at -0.35 V *vs* SCE and -0.80 *vs* SCE, respectively. This high value of ΔE (*i. e.* 0.45 V) is due to a low redox kinetic of the electrochemical couple on CF substrate. Moreover, the slight increase of ΔE along electropolymerization is due to film growth leading to an increase of the interface impedance. Another reason could be the pH change at the electrode surface, which generates protons during oxidation as proposed in the equilibrium (1) [38]. For potential higher than 0.8 V *vs* SCE, the formation of radical cations by electro-oxidation of NR leads to an over oxidation of the film decreasing its electrical properties [34, 39]. For this reason, the applied potential has been limited to 0.6 V *vs* SCE in this study.

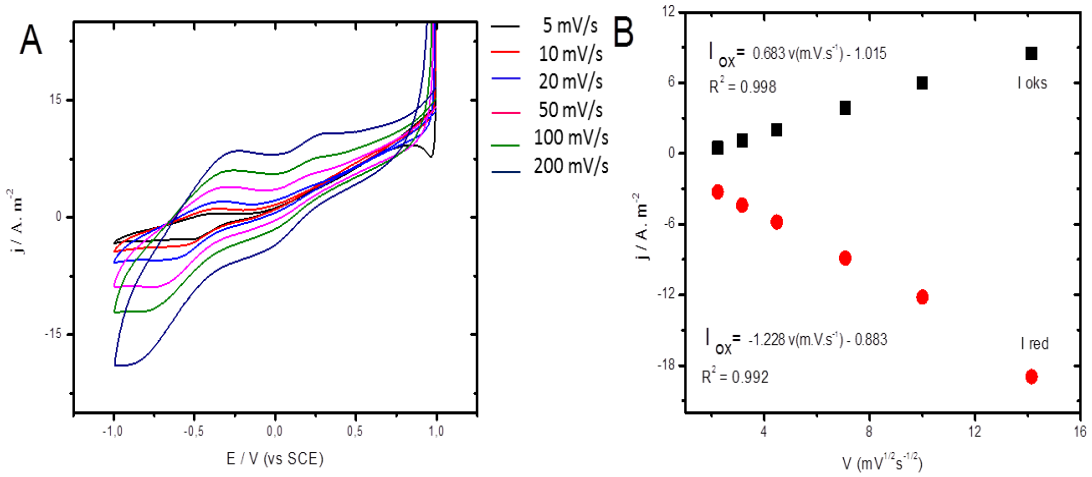


Figure 3. (A) Cyclic voltammogram of CF/PNR at different scan rates and (B) Dependence of anodic and cathodic peak currents *vs* square root of potential scan rate.

Fig. 2 (B) depicts the morphology of the PNR film deposited on the CF support. The inhomogeneous film structure is definitely due to the fibrous structure of felt. Nevertheless, EDX analysis has confirmed the PNR presence. After transfer in a monomer free solution, the electrochemical properties of CF/PNR were determined by cyclic voltammetry in a PB solution at pH 6 at potential scan rates varying from 5 to 200 mV/s (Fig. 3 (A)). At pH 6, PNR exists in the protonated form, $PNRH^+$, and is reduced to leuco-PNR, $PNRH_3^+$, following the equation (2) [37]:

$$PNRH^+ + 2H^+ + 2\,e \rightarrow PNRH_3^+ \qquad (2)$$

The redox peaks of $PNRH^+$ and $PNRH_3^+$ are identified at -0.8 V/SCE and -0,35V/SCE, respectively [37]. The reduction of $PNRH^+$ is associated to the insertion of proton within the polymer as counter ions and reversely for the oxidation step.

Increases of cathodic and anodic peak currents are linearly proportional to the square root of potential scan rate showing that the electrochemical redox process of CF/PNR is controlled by the diffusion of the counter ion, where there is no monomer in buffer solution [37].

3.2 Electropolymerization and characterization of CF/PNR entrapping yeast (CF/PNR-Y)

PNR has been synthesized following the procedure previously described in the experimental part with 2 vol.% of a suspension containing yeast at 2 g/100 mL. The aim of this part was to show the possibility of yeast embedding within the PNR film.

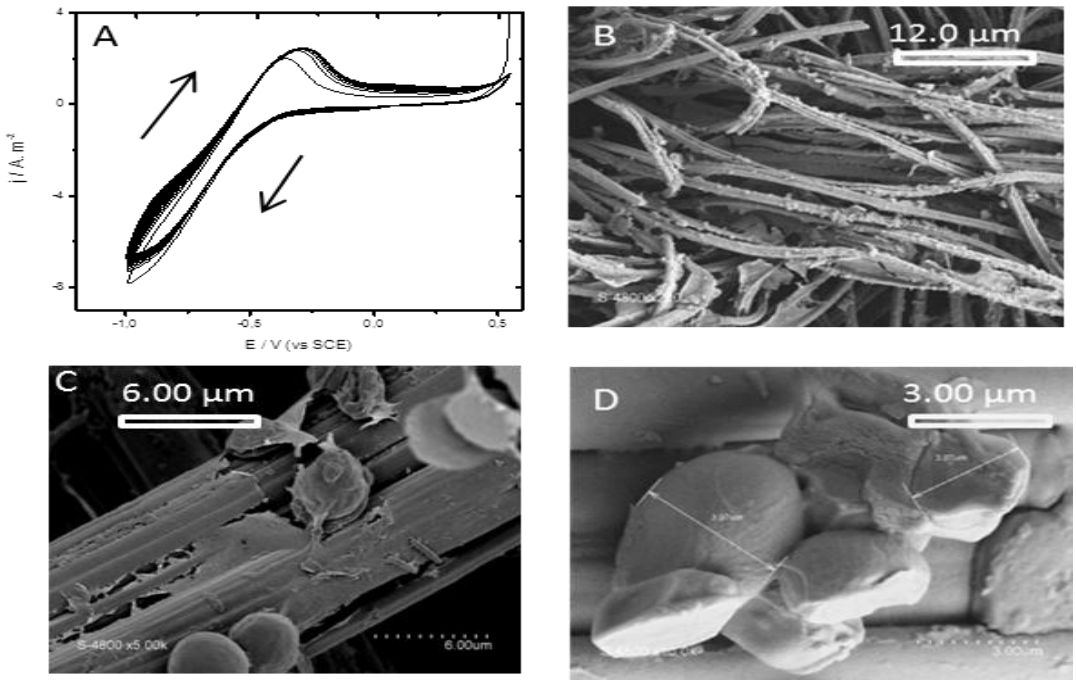


Figure 4. (A) Cyclic voltammogram of yeast entrapped in the PNR film (CF/PNR-Y) (15 cycles) and (B- to D) SEM images of the CF/PNR-Y membranes. Yeast was identified as cylindrical forms of around 3 microns of diameter.

Fig. 4 (A) shows the cyclic voltammogram of yeast entrapped in the PNR film (CF/PNR-Y) obtained after the electropolymerization of NR in the presence of yeast. It clearly appears that the current peak after 15 cycles, corresponding to the oxidation of PNR (-0.35 V/SCE) is lower when experiment is conducted in presence of yeast (Fig. 4 (A)) compared to electropolymerization in absence of yeast (Fig. 2 (A)). The decrease of the intensity is linked to a decrease of the electropolymerization rate definitively due to yeast adsorption. However, SEM micrographies

presented at Fig. 4 (C&D) clearly prove *Saccharomyces cerevisiae* is immobilized on the CF/PNR electrodes as cylindrical forms of around 3 μm.

3.3 Electrochemical characterization of yeast activity

Cyclic voltammetry was selected as a method to characterize the biologic activity of yeast towards the glucose oxidation. PNR transfers electrons from glucose to the electrode according to the scheme presented in Figure 5.

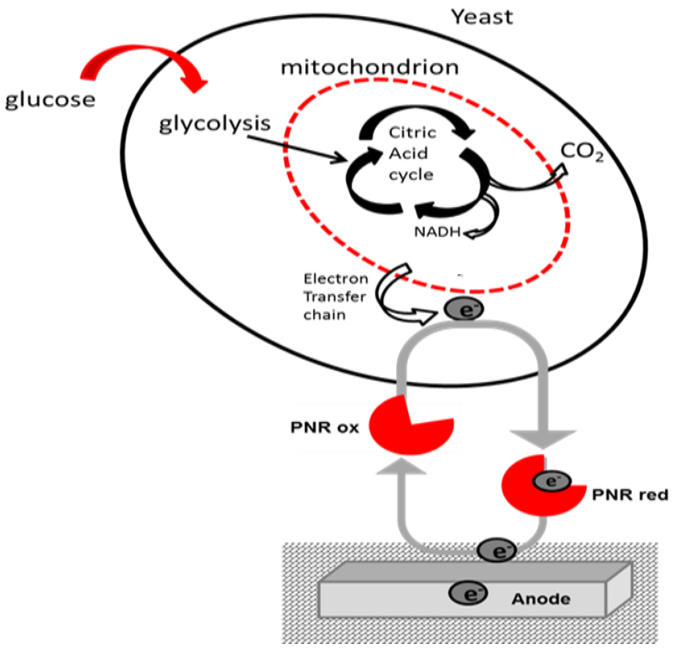


Figure 5. Scheme of the anaerobic fermentation pathway of *Saccharomyces cerevisiae* under anaerobic conditions showing the electrons transfer from PNR.

According to Fig.5, the electrocatalytic oxidation of glucose can be performed at the PNR film electrode thanks to NADH oxidation [33, 34]. Electrons produced are captured by the PNR under its oxidized form. In addition, the PNR film reduced form transferred electrons to anode. In anaerobic fermentation pathway, the recycling of NADH to NAD^+ is important to keep the glycolysis process continous [40, 41]. The use of redox mediators, switching between oxidized state (PNRox) and reduced state (PNR_{red}) is required in order to reach the electrons transfer chain located at the mitochondria within cytoplasm [42].

To prove first that NR is an efficient mediator, cyclic voltammogram was recorded on CF used as electrode in a solution containing NR (5 mM), yeast (2g/100mL) and with or without glucose (0.1 M).

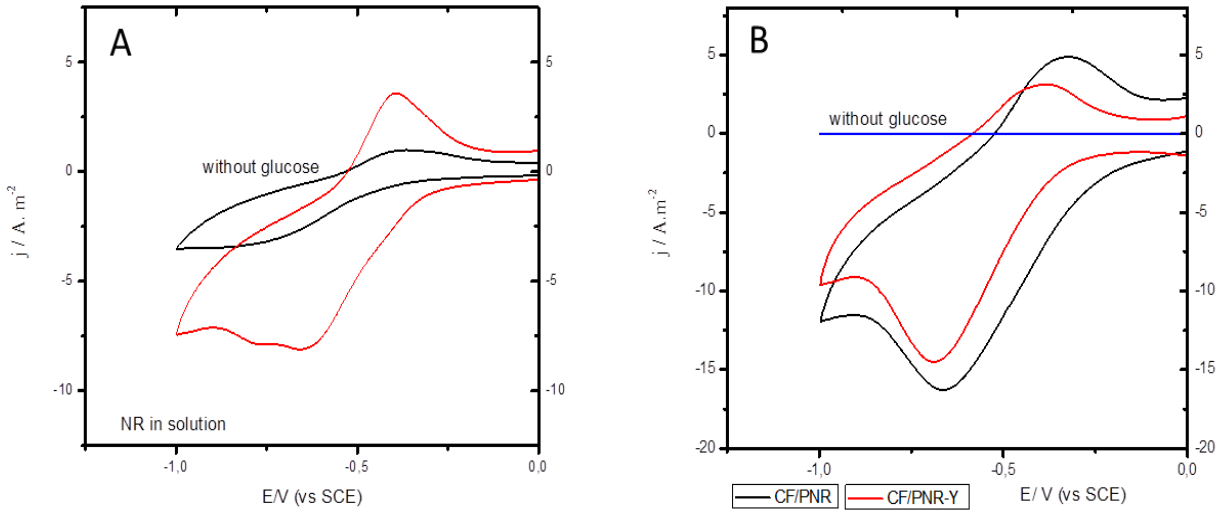


Figure 6. (A) Cyclic voltamogramm at CF electrode in NR (5 mM), yeast (2 g/100 mL) in PB pH 6, without glucose (black line) and with glucose (0.1 M) (red line) and (B) at CF/PNR(Y) electrode (red line) and at CF/PNR electrode (black line) in NR (5 mM) in PB pH 6, with glucose (0.1 M): Red line: yeast at 2 g/100 mL, black line: yeast immobilized at electrode.

The increase of the NR redox signal in presence of glucose observed in Fig. 6 (A) is due to the presence of *Saccharomyces cerevisiae* allowing the catalytic oxidation of glucose according to the pathway proposed in Figure 5. Moreover, Fig. 6 (B) compares the redox signals of the PNR film in presence of both the glucose and yeast, either entrapped within PNR (CF/PNR-Y) (red line) or in solution with yeast (CF/PNR) (black line). In both cases, redox currents relative to PNR are significantly higher for CV recorded with glucose compared to CV in absence of glucose. Such result clearly proves that PNR is an efficient glucose mediator either in solution or immobilized at the electrode.

Table 1 summarized the cathodic current density deduced from the CVs previously presented. It can be deduced that PNR is a more efficient mediator than NR in solution (used at 5 mM) because of the higher reduction current density obtained with CF/PNR compared to CF(Y-NR). When using PNR film as mediator, a lower cathodic current j_{red} is measured for yeast entrapped (14.33 $A.m^{-2}$ for CF/PNR-Y) than for yeast in solution (16.80 $A.m^{-2}$ for CF/PNR) that could be due to a higher quantity of *Saccharomyces cerevisiae* available for biocatalysis in this latter case.

Table 1. j_{red} max produced from different modified electrodes

System	j_{red} max ($A.m^{-2}$)
CF and NR and Y in solution (CF(Y-NR))	7.44
CF/PNR and Y in solution	16.80
CF/PNR-Y	14.33

To confirm the properties previously demonstrated by cyclic voltammetry, chronoamperometry experiments were carried out. Electrodes were polarized at 0.3V/SCE to ensure glucose oxidation. The resulting current j was monitoring during 2 hours after a stabilization time of 30 minutes.

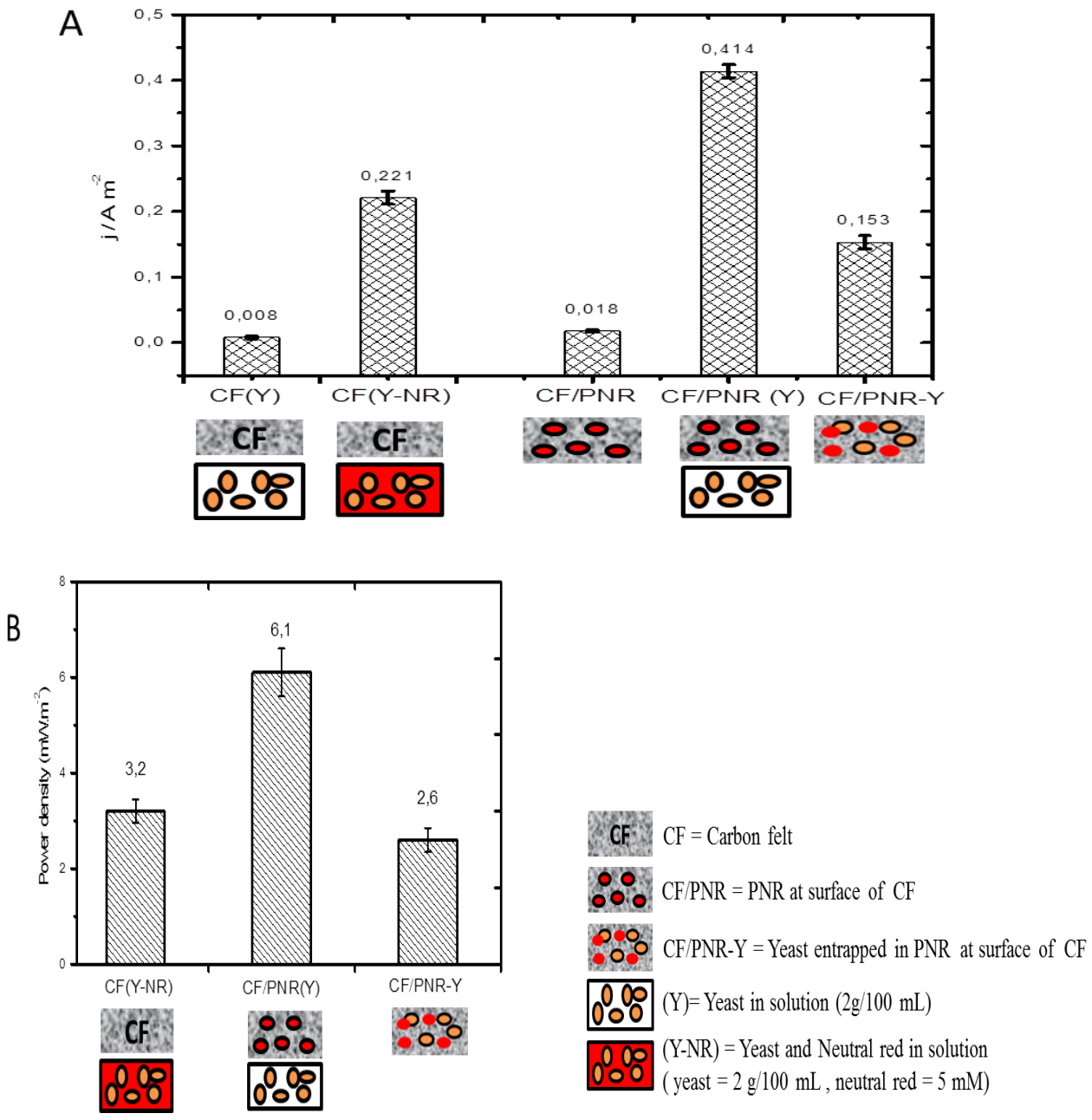


Figure 7. (A) Current density generated by the different anodes in a 0.1 M glucose solution and (B) Power density delivered at MFC.

From Fig. 7 (A), experiment conducted on CF with yeast in solution clearly proves the necessity to use a mediator since the delivered current density was very low (0.008 $A.m^{-2}$) for CF(Y). Indeed, the use of NR in solution at 5 mM allows electronic transfer, which leads to a current density

of 0.221 $A.m^{-2}$ at the electrode CF(Y-NR). Chronoamperometric measurements confirm that PNR is a better mediator than NR because of the higher current density produced at CF/PNR compared to NR solution (0.414 and 0.221 $A.m^{-2}$, respectively). Hence, it is clear that when yeast is entrapped within PNR (CF/PNR-Y), current is limited by the quantity of yeast: a current density of 0.153 $A.m^{-2}$ was determined against 0.414 $A.m^{-2}$ whether the yeast is entrapped or free in solution, respectively.

The low current delivered from CF/PNR-Y can be due to a low yeast quantity but also to a lower PNR quantity. We can estimate the amount of PNR deposited on surface using equation (3) :

$$n.m.F/M = \int_0^t I\, dt/v \quad \ldots\ldots\ldots\ldots (3)$$

where n is the number of electrons exchanged per mole involved in the redox couple of PNR (n = 2), m (g) is the mass of PNR electrogenerated, F is the Faraday constant (96.483 $C.mol^{-1}$), M the molecular weight of NR and *v* represents the scan rate. Applying eq. 3, CF/PNR gained 14.2 $\mu g/cm^2$, meanwhile CF/PNR-Y gained 9.2 $\mu g/cm^2$. It is then clear that the quantity of redox mediator is lower when yeast is entrapped within the film, which could conduct to a lower electron transfer kinetics.

Results presented Fig. 7 (B) showing the power density delivered at MFC, are in total accordance with the previous conclusions. Indeed, PNR definitely leads to a higher power density that NR if we compare results coming from CF(Y-NR) and from CF/PNR-Y. The quantity of yeast entrapped at electrode is also a limiting factor in MFC configuration as shown by the low power density generated by the anode CF/PNR-Y. Best performance in term of power density was measured at the load of 1 kΩ. In our case, after 8 days of running experiment, the maximum power density delivered by MFC was 6.1 ± 0.5 mW/m^{-2}. It is difficult to compare this value with the literature because MFCs operate under a large range of conditions: temperature, pH, presence and absence of electron acceptors, electrode surface area, reactor size and operation time. Note that the pH value is important to maintain the MFC system under the optimum operating conditions. In this present study, measurements have been done at pH 6 as a good alternative to get high performance of MFC [12].

4. CONCLUSION

NR and its polymerized form PNR have been successfully used as anodic electrons carriers for glucose oxidation. Electropolymerization of NR was conducted at carbon felt electrode by cyclic voltammetry between -1.0 and 0.55 V vs SCE. PNR has shown better properties for electrons transfer than NR through the generation of a higher current density in the presence of glucose and the yeast *Saccharomyces cerevisiae* in solution. Electropolymerization of NR was also conducted in the presence of yeast to entrap *Saccharomyces cerevisiae* within PNR. Best results were obtained using a film of PNR deposited on CF and working with yeast in suspension. The MFC built with the CF/PNR anode and working with yeast in suspension delivered a power density of 6 mW m^{-2} proving the interest to develop such devices.

ACKNOWLEDGEMENTS
This work was funded by Doctor Scholarships of Higher Education Ministry of Indonesian (DIKTI) Government in the framework of the Double Degree Indonesian France collaboration program and partially supported by European Membranes Institute (IEM) Montpellier France.

References

1. A.M.C. Potter, B. Character, N. Sep, Published by : The Royal Society Electrical Effects accompanying the Decormposition of Organic, 84 (2010) 260.
2. B.E. Logan, , *Nat. Rev. Microbiol*., 7 (2009) 375.
3. D.R. Lovley, *Nat. Rev. Microbiol.*, 4 (2006) 497.
4. D.R. Bond, D.R. Lovely, *Appl. Environ. Microbiol.* 69 (2003) 1548.
5. B.H. Kim, H.J. Kim, M.S. Hyun, D.H Park, *Microbiol Biotechn.*, 19 (1999) 127.
6. N.S. Malvankar, D.R. Lovley, *Curr. Opin. Biotechnol.*, 27 (2014) 88.
7. D. Prasad, S. Arun, M. Murugesan, S. Padmanaban, R.S. Satyanarayanan, S. Berchmans, et al., *Biosens. Bioelectron.* 22 (2007) 2604.
8. S.V. Raghavulu, R.K. Goud, P.N. Sarma, S.V. Mohan, Bioresource Technology, 102 (2011) 2751.
9. M. Rahimnejad, G.D. Najafpour, A.A. Ghoreyshi, M. Shakeri, H. Zare, *Int. J. Hydrogen Energy*. 36 (2011) 13335.
10. E.T. Sayed, Y. Saito, T. Tsujiguchi, N. Nakagawa, *J. Biosci. Bioeng.*, 114 (2012) 521.
11. Y.V. Hubenova, R.S. Rashkov, V.D. Buchvarov, M.H. Arnaudova, S.M. Babanova, M.Y. Mitov, *Ind. Eng. Chem. Res.*, 50 (2011) 557.
12. E.T. Sayed, T. Tsujiguchi, N. Nakagawa, *Bioelectrochemistry*, 86 (2012) 97.
13. Y. Zou, C. Xiang, L. Yang, L. Sun, F. Xu, Z. Cao, *Int. J. Hydrogen Energy*, 33 (2008) 4856.
14. A.Gunawardena, S. Fernando, F. To, *Int. J Molecular Sci.*, (2008) 1893.
15. S. Babanova, Y. Hubenova, M. Mitov, *J. Biosci. Bioeng.*,, 112 (2011) 379.
16. D.H.Park, M. Laivenieks, M.V. Guettler, M.K. Jain, J.G Zeikus, *Appl. Environ. Microbiol.*, 65 (1999) 2912.
17. D.A. Lowly, L.M. Render, J.G. Zeikus, D.H. Park, D.R. Lovley, *Biosens. Bioelectron.*.,21 (2006) 2058.
18. A.A.Karyakin, E.E.Karyakin, W. Schuhmann, , H.L. Schmidt, S.D. Varfolomeyev, *Electroanalysis*, 6 (1994) 821.
19. G.D Cruz, J. Abdulah, H. Sidek, S.A Rahman and A.N. Elina, *Sensor letter*, 9 (2011) 1.
20. H.P. Zhu, S.L. Mu, *Chinese Journal of Chem*., 19 (2001) 620.
21. Y. Hasebe, Y. Wang, K. Fukuoka, *J. Environ. Sc.*, 23 (2011) 1050.
22. Y.Dilgin, L. Gorton, G. Nisli, *Electroanalysis*, 19 (2007) 286.
23. K. Wang, Y. Liu, S. Chen, *J. Power Sources*. 196 (2011) 164.
24. D.H. Park, S.K. Kim, I.H. Shin, Y.J. Jeong, *Biotechnol. Lett.*, 22 (2000) 1301.
25. B. Lu, J. Bai, X. Bo, L. Yang, L. Guo, *Electrochim. Acta*, 55 (2010) 4647.
26. A.A.Karyakin, O.A Bobrova, E.E.Karyakin, *J. Electroanal Chem.*, 399 (1995) 179.
27. R. Pauliukaite, C.M.A. Brett, *Electroanalysis*, 20 (2008) 1275.
28. G. Inzelt, E. Csahok, *Electroanalysis*.,11 (1999) 744.
29. A.R. Gonçalves, M.E. Ghica, C.M. a. Brett, *Electrochim. Acta*, 56 (2011) 3685.
30. M.E. Ghica, R. Pauliukaite, N. Marchand, E. Devic, C.M. a Brett, *Analytica Chimica Acta*, 591 (2007) 80.
31. Y. Sun, B. Ye, W. Zhang, X. Zhou, *Analytica Chimica Acta* , 363 (1998) 75.
32. D. Benito, J.J. Garcı, *J. Electroanal. Chem.*, 446 (1998) 47.
33. S. Chen, K. Lin, *J. Electroanal. Chem.* , 511 (2001) 101.
34. D.G. Dilgin, D. Gligor, H.İ. Gökçel, Z. Dursun, Y. Dilgin, *Microchim. Acta*, 173 (2011) 469.

35. S. Tuncagil, D. Odaci, S. Varis, S. Timur, L. Toppare, *Bioelectrochemistry*, 76 (2009) 169.
36. C. Degran,L.L.Miller , *J. Am. Chem. Soc.*, 102 (1980) 5728.
37. R. Pauliukaite, M.E. Ghica, M. Barsan, C.M. a. Brett, *J. Solid State Electrochem.*, 11 (2007) 899.
38. C. Chen, Y. Gao, *Electrochim. Acta*, 52(2007) 3143.
39. C. Yang, J. Yi, X. Tang, G. Zhou, Y. Zeng, *React. Funct. Polym.*, 66 (2006) 1336.
40. D.L.Nelson, M.M.Cox, Priciples of biochemistry, 4th Ed, W.H. Freeman, New York (2005).
41. H. Feldmann, Yeast molecular biology – A short compendium on basic features and novel aspects, Adolf-Butenandt-Institute, university of Munich, Munich (2005).
42. O. Schaetzle, F. Barrière, K. Baronian, *Energy Environ. Sci.*, 1 (2008) 607.