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The optimization of the culture medium to design *Streptomyces* sp. M7 based impedimetric biosensors

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ABSTRACT

Electrochemical impedance has been widely used in microbiology in detection, quantifying and identifying microorganisms. *Streptomyces* strain M7 (*SM7*) is capable to grow in the presence of organochlorine pesticides (OCPs) as carbon source, using the amino acid asparagine as nitrogen source. This bacterial growth can be valuable and hence used in an impedimetric biosensor to detect and, eventually, quantify these pesticides. Since amino acids frequently used in culture media interfere in impedance determinations, this work presents an optimized design regarding the culture medium capabilities on the impedance response of *SM7* activity, as a first stage toward the development of an impedimetric biosensor to detect OCPs. With such a purpose, *SM7* growth in liquid or adhered to a solid media was measured by impedance in the presence of asparagine or $(NH_4)_2SO_4$ as nitrogen source and glucose as a model carbon source. Impedimetric *SM7* measurements were performed in the Quantibac instrument that can be employed to evaluate the metabolic behavior of microorganisms in different culture media using two fixed frequencies. We found that *SM7* used asparagine as carbon source in the absence of glucose. Since this consumption strongly contributed to the impedance signal, this is not a suitable medium to be used with an impedimetric biosensor. On the other hand, $(NH_4)_2SO_4$ appears as an ideal medium because it supports *SM7* growth in liquid and solid media without interfering the impedimetric detection. This simple and cost-effective technique combined with a spore-based platform is a promising strategy to design OCPs bacteria-based biosensors.

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1. Introduction

Impedimetric detection combines the analysis of the resistive and capacitive (or inductive) properties of a system in response to a small amplitude sinusoidal excitation signal [1,2] applied between two electrodes. Since the used voltage is low, impedance measurement does not disturb the properties of the system being measured, which then makes it an ideal technique for cell culture monitoring [3]. This technique has been applied in the detection, quantification and even identification of microorganisms in liquid [4–7] and solid [8,9] culture media [10,11]. This technique was also used for the detection of bacterial cells by using interdigitated array microelectrodes [7,12] as well as to study the effects of bacterial adhesion to electrodes [13–15]. The growth of microorganisms increases the conductivity of the medium by converting uncharged or weakly charged substances present in the culture medium (yeast, peptone, sugar, etc.), into highly charged substances (amino acids, aldehydes, ketones, acids, and other metabolic products) [16]. This change in the ionic composition due to the bacterial activity at the immediate neighborhood of the electrode also changes the interface capacitance. Consequently, this cheap and non-destructive technique works by measuring the impedance change caused by modifications in the medium resistance and in the interface reactance, produced by the growth and metabolism of microorganisms [17–21].

Bacterial biosensors coupled to impedimetric detection can be classified into two types, depending on the location of the bacterial cells in the experimental setup. One group works by measuring the impedance change caused by the interaction between the

Abbreviations: SM7, Streptomyces strain M7; MM, liquid-defined medium; MMAsn, liquid-defined medium supplemented with asparagine; $MM(NH_4)_2SO_4$, liquid-defined medium supplemented with ammonium sulfate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MM, minimal *Aspergillus* medium; SC, starch-casein agar.

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analyte and bacteria-modified electrodes [14,15]. The detection principle of the other type is based on measuring the metabolites produced by bacterial cells as a result of growth in the presence of the analyte [7,22,35].

This last method relies on determining the changes in electrical impedance of a culture medium resulting from the bacterial growth. In this case, the culture medium not only supports the bacteria growth but also provides high, non-interfering signals to the overall impedance or its components [23]. In this case, the sensitivity of the impedance detection strongly depends on designing an appropriate culture medium.

Streptomyces strain M7 (*SM7*) is capable to grow in the presence of organochlorine pesticides (OCPs) as carbon source, using asparagine (Asn) as nitrogen source [24–27]. This bacterial growth can be valuable used in an impedimetric biosensor to detect and, eventually, quantify these pesticides. Under the appropriate conditions, the growth and metabolism of *SM7* in the presence of the pesticide as the carbon source may result in chloride ion release changing the medium resistance and/or may produce metabolites that modify the interface capacitance. An impedimetric biosensor is an inexpensive and simple alternative to the American Public Health Association recommended method (gas chromatography with electron capture) to detect OCPs [28] which is expensive and needs highly trained personnel. A key issue to design and develop such biosensor is the optimization of the culture medium in which the carbon source, acting as the analyte, will be added. For instance, *SM7* has also the capability to grow in liquid media containing only Asn as carbon and nitrogen sources [50]. Considering that amino acids contribute to biomass growth by augmenting the pool of available amino acids and by boosting the tricarboxylic acid cycle [29], Asn may interfere in the impedimetric detection of the pesticide when coupled to a *SM7* based biosensor.

As a first stage toward the development of an impedimetric biosensor to detect OCPs, this work presents an optimized design regarding the culture medium capabilities on the impedance response of *SM7* activity. With such a purpose, *SM7* growth in liquid or solid media was measured by using the commercial impedancimetric equipment Quantibac (TecnoVinc S.R.L.) in the presence of Asn or $(NH_4)_2SO_4$ as two different nitrogen sources and glucose (Glu) as a model carbon source, acting as the analyte. This equipment uses a two frequencies impedance method, and can be employed in microbiology to detect, quantify or evaluate the metabolic behavior of microorganisms in different culture media. Using these two frequencies (20 Hz and 20 kHz), the two contributing components (medium resistance and interface reactance) of the impedance can be distinguished. While the medium component is independent of the frequency in this range, the interface component is frequency-dependent. At low frequencies, the interface component is very high comparing to the medium resistance, so it is the main component that contributes to the total impedance. At high frequencies (20 kHz in the present case), the interface reactance becomes very low, and its contribution to the total impedance is negligible. This way, the only component of the total impedance is the medium resistance. Therefore, the changes in the interface reactance and in the medium resistance during bacterial growth can be separately detected by impedance measurement at these two frequencies [30,31]. To the best of our knowledge, this is the first attempt to design an impedimetric, bacteria-based biosensor to detect OCPs using the resistive and capacitive components of the impedance.

2. Materials and methods

2.1. Reagents

L-Asparagine (Asn) and $(NH_4)_2SO_4$ were purchased from Sigma–Aldrich, D-glucose (Glu), $FeSO_4{\cdot}7H_2O$and K_2HPO_4 from J.T. Baker, starch and $MgSO_4{\cdot}7H_2O$ from Cicarelli, casein from Calbiochem and agar-agar from Britania. These chemicals were reagent grade and used without further purification. Glu consumption was spectrophotometrically determined with a commercial kit (Wiener lab, Argentina). All solutions were prepared with ultrapure water (ρ = 18 MΩ cm) from a Millipore-MilliQ system.

2.2. Bacteria strain and culture media

A characterized strain, *Streptomyces* sp. M7 [24,32], isolated from Salí river (Tucumán, Argentina) was used for this work. This strain was immobilized on a solid culture medium (starch–casein agar, SCA) or suspended in a liquid-defined medium (MM). SCA contained (in $g\,L^{-1}$): starch, 10.00; casein, 1.00; K_2HPO_4, 0.50; agar, 15.00 [33] and MM contained (in $g\,L^{-1}$): K_2HPO_4, 0.50; $MgSO_4$, 0.20; $FeSO_4$, 0.01. MM was supplemented with (in $g\,L^{-1}$): Asn, 0.50 (MMAsn) or $(NH_4)_2SO_4$, 2.00 (MM$(NH_4)_2SO_4$) as nitrogen sources and Glu, 10.00, as carbon source. All culture media were adjusted to pH 7.00 and sterilized by autoclaving for 20 min at 120 °C. Glu was sterilized by filtration (0.22 μm pore size Millipore filter) and then added aseptically to the autoclaved medium.

2.3. Equipments

Impedance measurements were performed with the commercial equipment QUANTIBAC [34–37]. The instrument simultaneously measures impedance and turbidity in a culture cell. It is composed of two incubators with variable temperatures independently controlled. The cells are glass bottles of 10.0 mL capacity with two stainless steel electrodes (electrode area: 0.31 cm^2; distance between electrodes: 0.8 cm). The bipolar impedance between these two electrodes is measured at 20 Hz (1 Vpp, 5 μA) and 20 kHz (1 Vpp, 1 mA) in order to separate the medium and interface components [36]. These two components are calculated through an equivalent electric circuit that provides the information of the interface reactance (X_i) at 20 Hz, while high frequency impedance collects the information about medium resistance (R_m). The equipment has 30 independent channels and measures these two electrical and one optical parameters in each channel. Impedance information is presented as time course curves in percentage terms for R_m (%R_m) and X_i (%X_i). These values are directly provided by the equipment and calculated as the relative percentage at any given incubation time to the corresponding initial value.

A UV-1601 Shimadzu spectrophotometer equipped with PC-interface and analytical software was used for the spectrophotometric determinations of Glu consumption.

2.4. Experimental

Cultivation conditions included spore suspensions and adhered bacteria on SCA. In the first case, Quantibac cells containing 6.0 mL of MM were inoculated with a 1.0 mL aliquot of *SM7* spore suspensions of concentration 160×10^5 CFU mL^{-1} and culture at 30 °C, without shaking, during 4 days. MM was used as culture medium without nitrogen source and MMAsn and MM$(NH_4)_2SO_4$ as nitrogenous culture media. To evaluate the additional effect of a carbon source, Glu was added to the different liquid culture media before mentioned.

Adhered bacteria assays were performed on Quantibac cells containing previously adhered *SM7* on SCA by surface dissemination, and then culturing with the corresponding liquid MM added into the cells. Both cultivation conditions (adhered and suspended) were carried out at 30 °C, without shaking, during 7 and 4 days, respectively. The appropriate non-inoculated controls were also prepared. Each experiment was carried out by duplicate and the results are informed as the arithmetic means of six

Quantibac independent channels. Bacterial activity was evaluated by changes in electrical impedance in the liquid medium and in the electrode–electrolyte interface.

In addition, supernatant samples of centrifuged cultures ($9900 \times g$, 10 min) were used to determine residual glucose after 4 days of cultivation. Glu determination was carried out by an enzymatic assay with a commercial kit based on glucose oxidation by glucose oxidase-peroxidase [38]. Quantitative analysis of samples was performed by using appropriate glucose calibration standards and also considering the Glu sorption percentage in SCA. All the manipulations were made under sterile conditions. All measurements were performed at 25 °C.

Statistical analyses were performed with Infostat software (developed by Universidad Nacional de Córdoba [39]. An ANOVA three ways was used for data variance analysis, considering significant a probability level of $p < 0.05$ using DGC technique [40] to perform multiple comparisons of variables.

3. Results

Although impedimetric measurements by Quantibac [34–37] are mostly used to quantify bacteria concentration in a culture medium, they can also be applied to determine the presence or concentration of a given analyte in solution. In this last case, the impedance signal depends on the metabolites produced by bacterial cells as a result of growth in the presence of the analyte (the model carbon source, Glu, for the matter of this study). Figs. 1 and 2 show typical impedance profiles of bacterial growth [4,34–37] in liquid culture media. Both figures present the results of the medium resistance percentage ($\%R_m$) as a function of *SM7* cultivation time in panel (a) and the interface reactance percentage ($\%X_i$) curves in panel (b). Although these impedance components were followed during 4 days, the figures show only the results obtained during the first 2 days because, in most cases, steady values were reached in this period of time. Reference cells (i.e. non-inoculated cells) did not show changes in $\%R_m$ or $\%X_i$ during the 4 cultivation days indicating that in the absence of bacteria there was not any measurable change. From these bacterial growth curves, different analytical responses can be used to detect the presence of a particular analyte in solution through the *SM7* metabolic pathways: (1) the detection time; (2) $\%R_m$ and $\%X_i$ steady values; and (3) the features of the complete impedance profiles. The detection time (t_d), defined as the time at which the growth curves present the inflection point, is usually used to quantify the initial number of bacteria [41,42] in a given culture medium. However, in this study the initial number of bacteria was kept constant (160×10^5 CFU mL^{-1}) and the value of t_d was evaluated as a possible parameter to characterize the metabolic response of *SM7* in the different culture media. Moreover, the algorithm to calculate t_d from a given profile is included in the Quantibac instrument [37], making this parameter a simple analytical response. The average steady values of $\%R_m$ and $\%X_i$ and the corresponding t_d values measured from triplicate experiments (3 Quantibac cells) measured in two independent runs ($n = 6$) are given in Table 1 for the different culture media.

Fig. 1 compares the impedance profile of *SM7* in MM (circles) and MM supplemented with Asn (triangles) in the absence (solid symbols) and the presence (open symbols) of Glu. It is important to emphasize that even though Asn is usually added to the culture medium as a nitrogen source, for *SM7* it also acts as a carbon source in the absence of any other one [52]. *SM7* growth curves in MM (both in the presence and the absence of Glu) did not show any change in $\%R_m$. On the other hand, the measurements performed in MMAsn with and without Glu indicated an increase of $\%R_m$ during the first 24 h of cultivation, reaching different steady values depending on the carbon source (Asn or Glu). Hence, Asn used as a nitrogen source interferes the steady $\%R_m$ values achieved by the carbon source. Analysis of the $\%X_i$ *SM7* growth curves in MM with Glu showed a gradual increment after 24 h which reached a steady value at the end of the 4 experimental days. $\%X_i$ curve of *SM7* in MMAsn also indicated *SM7* activity regardless the presence of Glu in the culture medium. In this case, the steady values were achieved between 20 h and 30 h with a decline phase in the *SM7* growth curve in MMAsn without Glu. As it was the case with $\%R_m$, steady $\%X_i$ values also depended on the carbon source and Asn was interference to detect Glu.

Table 1
Steady values of the medium resistance ($\%R_m$) and the interface reactance ($\%X_i$) percentages and the detection time (t_d) measured from *SM7* growth curves performed in different culture media.

SM7 in:	$\%R_m$	t_d (h)	$\%X_i$	t_d (h)
MM	0	–	1 ± 3	–
MM + Glu	0	–	15 ± 2	17 ± 1
MM + Glu (adhered to SCA)	0	–	14 ± 2	13 ± 1
MMAsn	28 ± 4	13 ± 2	11 ± 3	14 ± 2
MMAsn + Glu	6 ± 1	12 ± 2	21 ± 2	11 ± 1
MMAsn + Glu (adhered to SCA)	8 ± 1	14 ± 2	17 ± 2	7 ± 1
$MM(NH_4)_2SO_4$	0	–	3 ± 3	–
$MM(NH_4)_2SO_4$ + Glu	1 ± 1	–	21 ± 2	13 ± 1
$MM(NH_4)_2SO_4$ + Glu (adhered to SCA)	2 ± 2	–	20 ± 2	9 ± 1

The values of t_d were calculated from $\%X_i$ curves when *SM7* was in MM whereas both profiles ($\%R_m$ and $\%X_i$) were used with MMAsn in the absence and the presence of Glu. Table 1 shows that t_d values were rather unaffected by the carbon source. However, the absence of Asn significantly increased this value. These results clearly demonstrate that Asn interferes the impedance bacterial growth curves of Glu. Therefore, MMAsn is not an appropriate liquid culture medium to be used with *SM7* impedimetric biosensors based on the degradation of a carbon source. On the other hand, MM does not represent interference because the t_d value in the absence of the nitrogen source is significantly higher than in the Asn presence. Therefore, a nitrogen source is necessary to measure the bacterial growth in the presence of a carbon source with shorter experiments. As an alternative nitrogen source, MM was supplemented with $(NH_4)_2SO_4$. Fig. 2 shows impedance records of *SM7* in MM (circles) or $MM(NH_4)_2SO_4$ (triangles) with and without Glu. $\%R_m$ values were found to be near zero during the 4 cultivation days in the absence and presence of Glu. On the other hand, $\%X_i$ results indicated that *SM7* in MM or $MM(NH_4)_2SO_4$ only showed growth curves when Glu was added to the culture media. The steady $\%X_i$ (reached after 24 h of cultivation) and t_d values in $MM(NH_4)_2SO_4$ were similar to those measured in MMAsn with Glu. Therefore, the modified culture medium with $(NH_4)_2SO_4$ as nitrogen source is well suited to *SM7* growth and does not interfere with impedance determinations. Steady $\%R_m$ and $\%X_i$ values would be appropriate analytical parameters to detect an analyte (that acts as a carbon source for *SM7*) present in solution, providing that no other medium compound produces an impedimetric signal as Asn does.

In order to evaluate the complete impedance profiles as a measurement of the presence of a particular carbon source, a statistical analysis on the values of $\%R_m$ and $\%X_i$ as a function of the cultivation time was performed every 12 h with triplicate experiments (3 Quantibac cells) measured in two independent runs ($n = 6$). Fig. 3 shows $\Delta\%R_m$ (panel a) and $\Delta\%X_i$ (panel b) values of *SM7* in the different liquid culture media. $\Delta\%R_m$ and $\Delta\%X_i$ values were calculated by subtracting the corresponding reference signal ($\%R_{m(Reference)}$ or $\%X_{i(Reference)}$) to the sample signal ($\%R_{m(Sample)}$ or $\%X_{i(Sample)}$). As expected, $\Delta\%R_m$ values were only measurable with *SM7* in MMAsn and there were statistically significant differences ($p < 0.05$) in $\Delta\%X_i$ when Glu or Asn was consumed by *SM7* as the carbon source. Hence, both $\Delta\%R_m$ and $\Delta\%X_i$ measured in $MM(NH_4)_2SO_4$ can be used to

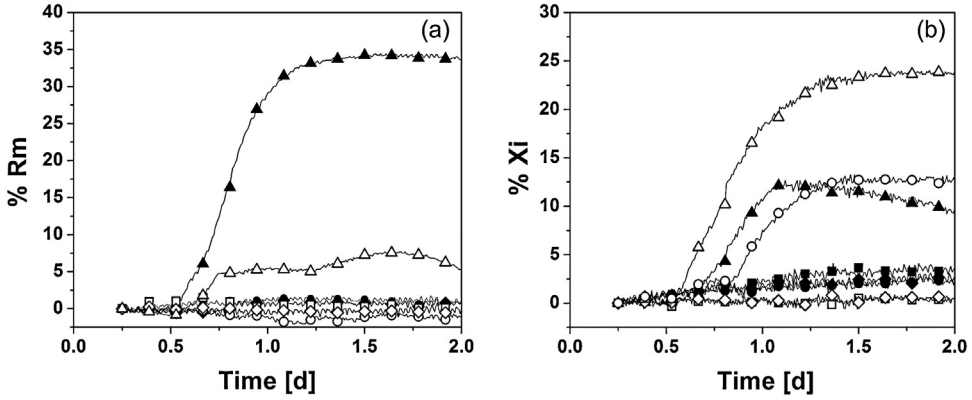

Fig. 1. (a) Medium resistance percentage ($\%R_m$) and (b) interface reactance percentage ($\%X_i$) curves of *SM7* in (●) MM and (▲) MMAsn. Reference (non-inoculated) culture media: (■) MM and (♦) MMAsn. Open symbols show culture media supplemented with Glu 10.0 g L^{-1}. Lines only point out data trend.

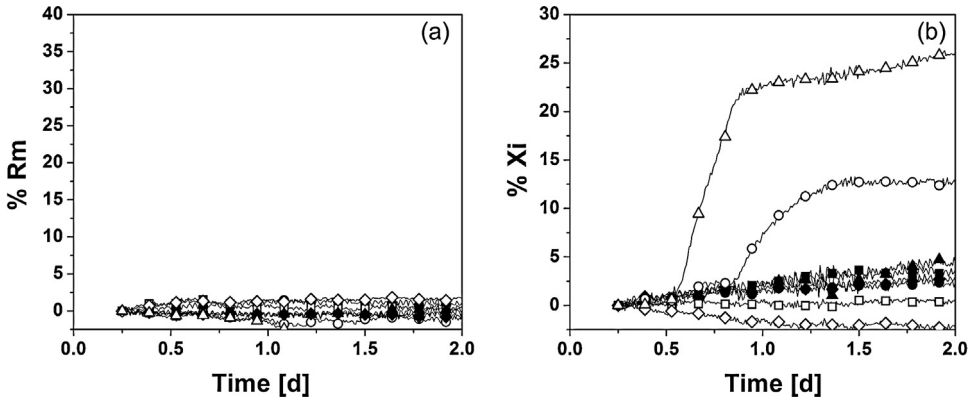

Fig. 2. (a) Medium resistance percentage ($\%R_m$) and (b) interface reactance percentage ($\%X_i$) curves of *SM7* in (●) MM and (▲) $MM(NH_4)_2SO_4$. Reference (non-inoculated) culture media: (■) MM and (♦) MMAsn. Open symbols show culture media supplemented with Glu 10.0 g L^{-1}. Lines only point out data trend.

distinguish different carbon sources even at low cultivation time. This statistical analysis also confirms that Asn behaves interfering when *SM7* activity is evaluated by impedance measurements.

Recalling that solid medium is more appropriate to design bacteria based biosensors because it is easy to handle and portable compared to liquid medium, the activity of adhered *SM7* was also evaluated. Prior to impedance measurements, the bacterial activity toward Glu consumption was determined in the presence of either Asn or $(NH_4)_2SO_4$ as nitrogen source. Fig. 4 shows the direct measurement of the activity (as cumulative percentage of Glu consumption) of *SM7* adhered to SCA medium in contact with MMAsn (solid symbols) and $MM(NH_4)_2SO_4$ (open symbols) culture media at different incubation times. The bacterial activity slowly increased up to the third incubation day and continued until complete depletion of the carbon source after 8 incubation days. The activity profile did not depend on the composition of the culture medium; hence, changing the nitrogen source did not disrupt *SM7* activity even in the adverse condition when adhered to a solid culture medium.

SM7 activity was also followed by impedance with the bacteria adhered to SCA medium in contact with different liquid media (where the electrodes were immersed). Fig. 5 shows impedance records of adhered *SM7* immersed in MM (circles), MMAsn

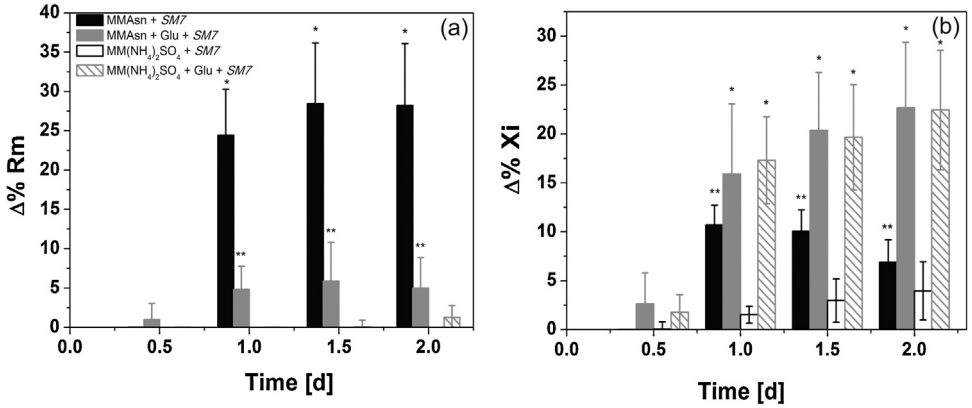

Fig. 3. Percentage variation of changes in (a) medium resistance ($\Delta\%R_m = \%R_{m(Sample)} - \%R_{m(Reference)}$) and (b) interface reactance ($\Delta\%X_i = \%X_{i(Sample)} - \%X_{i(Reference)}$), for *SM7* growing in MMAsn and $MM(NH_4)_2SO_4$, with and without Glu 10.0 g L^{-1}. (■) MMAsn + *SM7*, (▩) MMAsn + Glu + *SM7*, (□) $MM(NH_4)_2SO_4$ + *SM7* and (○) $MM(NH_4)_2SO_4$ + Glu + *SM7*.

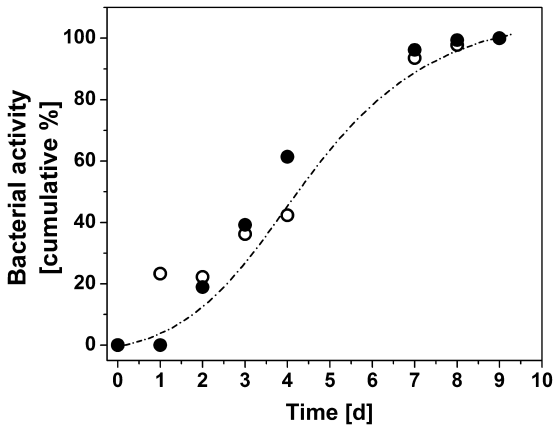

Fig. 4. Bacterial activity of adhered *SM7* to SCA stated as cumulative percentage of Glu consumption as a function of the incubation time. (●) MMAsn, (○) $MM(NH_4)_2SO_4$. Line only points out data trend.

(squares) or $MM(NH_4)_2SO_4$ (triangles) supplemented with Glu during the first 2 incubation days. Panels a and b display $\%R_m$ and $\%X_i$, respectively. Reference cells (i.e. non-inoculated cells) did not show changes in $\%R_m$ and $\%X_i$ values during the 4 incubation days. Table 1 displays the steady $\%R_m$ and $\%X_i$ and t_d values. As it was the case with *SM7* suspended in liquid media, an increase in $\%R_m$ was only observed with MMAsn reaching similar steady and t_d values. Moreover, in the presence of $(NH_4)_2SO_4$, $\%R_m$ was found to be near zero during the 4 days. Hence, Asn also interfered $\%R_m$ measurements when *SM7* was adhered to a solid medium. On the other hand, $\%X_i$ growth curves were observed in the three culture media, reaching similar steady values after 36 h of incubation in the presence of a nitrogen source. Recalling that the presence of Glu as the carbon source produces the same steady $\%X_i$ value, it represents a well suited analytical parameter to detect the presence of a particular carbon source. Regarding t_d, it is noticeable that adhered bacteria presented significantly smaller values for all liquid media than free suspended cells. Therefore, besides the usual advantages of using solid medium to design bacteria based biosensors, *SM7* adhered to SCA also allows reducing the determination time. Fig. 6 compares $\Delta\%R_m$ (panel a) and $\Delta\%X_i$ (panel b) values of *SM7* suspended in liquid and adhered to solid media. *SM7* in MMAsn produced the same $\Delta\%R_m$ signals up to the 36 h of cultivation, which resulted in the same t_d values for both media. On the other hand, $\Delta\%X_i$ features in MMAsn or $MM(NH_4)_2SO_4$ were different, specially at short incubation times (less than 24 h). Adhered *SM7* responded faster than the suspended bacteria.

4. Discussion

Designing an appropriate culture medium for bacteria is of critical importance since its composition significantly influences the optimum growth, activity, products yield [43–45], and morphology [46] of the microorganisms. Particularly, there are several reports [47–49] about the effect of the nature and type of carbon, nitrogen or phosphorus sources and trace elements on cell

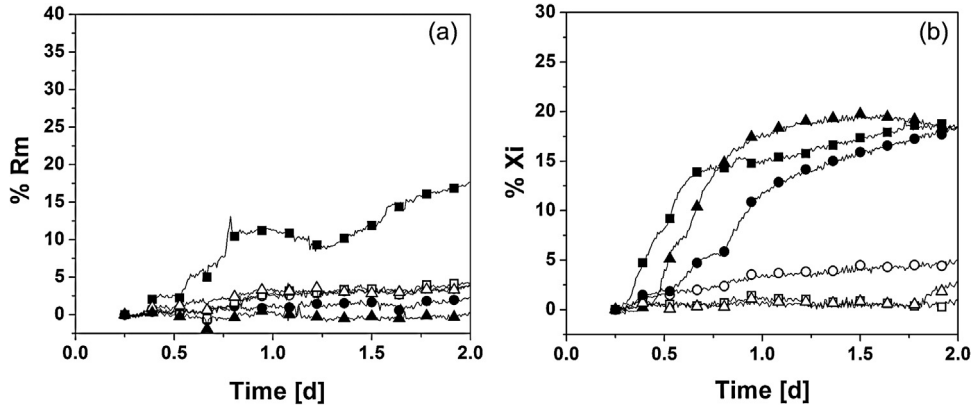

Fig. 5. (a) Medium resistance percentage ($\%R_m$) and (b) interface reactance percentage ($\%X_i$) curves of adhered *SM7* to SCA in (●) MM, (■) MMAsn and (▲) $MM(NH_4)_2SO_4$ in the presence of Glu. Reference (non-inoculated) culture media (with Glu): (○) MM; (□) MMAsn; and (△) $MM(NH_4)_2SO_4$. Lines only point out data trend.

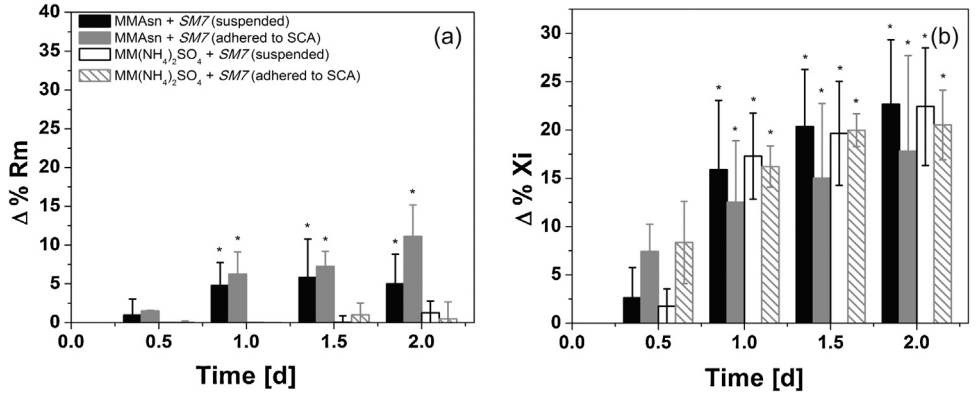

Fig. 6. Percentage variation of changes in (a) medium resistance ($\Delta\%R_m = \%R_{m(Sample)} - \%R_{m(Reference)}$) and (b) interface reactance ($\Delta\%X_i = \%X_{i(Sample)} - \%X_{i(Reference)}$), for *SM7* growing in MMAsn and $MM(NH_4)_2SO_4$, with Glu 10.0 g L^{-1}. (■) MMAsn + *SM7*, (▩) MMAsn + *SM7* (adhered to SCA), (□) $MM(NH_4)_2SO_4$ + *SM7* and (○) $MM(NH_4)_2SO_4$ + *SM7* (adhered to SCA).

growth, biotransformation, and antibiotic biosynthesis by *Streptomyces*. Therefore, to achieve maximum bacterial activity and products yield it is necessary to optimize nutrients and environmental conditions. This is also the case, when *SM7* is used to design a bacterial biosensor coupled to impedimetric detection. In fact, Asn mostly used as a nitrogen source for optimum *SM7* growth [49–51], strongly contributes to the impedance signal of the carbon source which represents the analyte in the medium. Therefore, MMAsn is not a suitable medium to couple a *SM7* biosensor to the Quantibac instrument. On the other hand, $MM(NH_4)_2SO_4$ appears as an ideal medium because it supports *SM7* growth without interfering the impedimetric detection of the analyte. Furthermore, $MM(NH_4)_2SO_4$ is also a very good nitrogen source for *SM7* adhered to SCA, combining the advantages of the durability and resilience of bacterial spores, and the quickness and easy to use of the impedance technique. Accordingly, *SM7* adhered to SCA immersed in $MM(NH_4)_2SO_4$ represents the optimum culture medium to determine the presence of a carbon source with an impedimetric biosensor.

In order to understand Asn interference in a more general picture, it is necessary to consider the processes that affect the impedance contributions. Continuous increments in $\%R_m$ (Fig. 1 panel a) point out that bacterial activity modifies the concentration of the ionic species in the culture medium, perceived as resistance changes. When Asn is consumed as carbon and nitrogen sources in the absence of an additional carbon source [52], the ionic composition of the medium is strongly changed due to the *SM7* metabolic activity. In the presence of Glu, $\%R_m$ changes slightly suggesting that only the presence of Asn modifies the concentration of the ionic species in the culture medium. This is a surprising result considering that in the presence of another carbon source, Asn consumption by *SM7* is very low [52]. Hence, the small amount of metabolites produced by consuming Asn is enough to change $\%R_m$. Moreover, the presence of Glu in $MM(NH_4)_2SO_4$ does not change $\%R_m$ (Fig. 2 panel a), indicating that this metabolic bacterial activity does not modify the concentration of the ionic species in the medium. Therefore, the change in $\%R_m$ is only due to the presence of Asn, revealing different bacterial metabolic activity for each carbon source (Glu or Asn). In view of using a *SM7* impedimetric biosensor to detect OCPs, the ability of the medium resistance component of the impedance to differentiate one carbon source from other one is very promising because a possible bacterial pathway to degrade these pesticides may release chloride ions to the medium. For this reason, it is mandatory to remove Asn interference.

Changes in $\%X_i$ are more difficult to interpret because the interface reactance contains contributions due to the geometry (roughness) of the electrodes and also to the electrochemical characteristics of the interface. A typical nutrient broth is a complex mixture of organic and inorganic ions and compounds. Bacterial metabolism can modify the double-layer capacitance because of the induced changes in the ionic diameters, in the charges and in the ionic concentrations [53]. In addition, bacterial attachment to the electrodes also could modify the double layer capacitance [13–15]. This is not the case of *SM7* because the impedance response measured with adhered bacteria to SCA show the same profile features than suspended bacteria in liquid media (Fig. 6), except for the first hours of cultivation. However, growth $\%X_i$ curves indicate changes in the electrodes interfaces due to diverse responses in the equipment produced by *SM7* metabolism with different culture media (Figs. 1, 2 and 5 panel b). These profiles show different bacterial metabolism when: (a) Asn behaves as the nitrogen or carbon sources, (b) Asn or Glu is the carbon source, (c) the nitrogen source is not added, (d) *SM7* is adhered at short incubation time. These results indicate that modifications in double layer capacitance are not due to the bacterial attachment to the electrode but to the change in ionic composition of the double layer. It is important to point out that $\%X_i$ values appear as the most sensitive analytical response regarding the presence of a particular carbon source in the medium. Therefore, the metabolites produced by *SM7* with OCPs as a carbon source may also affect the interface reactance of the impedance.

Biosensors designs should fulfill essential features such as portability, cellular viability, reuse of the cells, long-term storage and fast response [54]. These requirements are best accomplished with adhered than free suspended cells. Moreover, bacterial spores are known to be able to be quiescent and preserve bacterial DNA for long periods of time even in harsh environmental conditions [55]. Date et al. [56] developed spore-based sensing systems to detect arsenic and zinc; they also demonstrated the ability of spores to preserve the analytical characteristics of whole-cell sensing systems for extended periods of time. The results of this study confirm that adhered spores germinate to fully active cells responding faster to analytes than suspended bacteria, as shown by t_d values in Table 1. Impedimetric sensing perceived *SM7* activity only in 10 h of cultivation or less. Therefore, the proposed biosensor design, combining an adhered spore-based platform with impedimetric sensing, takes advantage of the durability and resilience of bacterial spores, and the quickness and easy to use of the impedance technique. This strategy cost-effectively satisfies biosensors requirements and could be successfully employed in future biosensors developments.

5. Conclusions

SM7 metabolism of different nitrogen and carbon sources can be study simply and cost-effectively by impedance changes using just two fixed frequencies. Thus, impedance is an efficient and fast method to analyze the differences in the bacterial metabolism respect to the diverse nutrients sources. Adhered *SM7* to SCA in $MM(NH_4)_2SO_4$ represents a well suited growth medium to be used in the impedimetric detection of carbon sources. Furthermore, this technique combined with a spore-based platform is a promising strategy which effectively accomplished the requirements to design OCPs bacteria-based biosensors.

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