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Assessment of growth of *Fusarium solani* by cyclic voltammetry and possible bioanalytical applications

Sreenath Subrahmanyam†,*[a] Kumaran Shanmugam,[a] V. Murali Madhav,[b] M. Murugesan,[c] T. V. Subramanian[a] and D. Jeyakumar[c]

[a] *Department of Chemical Engineering, Alagappa College of Technology, Anna University, Madras 600 025. Tamil Nadu State, India*
[b] *Division of Biosensors, Central Food Technological Research Institute, (CFTRI), (Council of Scientific and Industrial Research), Mysore, Karnataka State, India*
[c] *Electrodics and Electrocatalysis Division, Central Electrochemical Research Institute, (CECRI), (Council of Scientific and Industrial Research), Karaikudi 630 006. Tamil Nadu State, India*

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Fusarium solani, the fungus isolated from polyurethane waste scraps, was studied for its voltammetric response. The peak current values were obtained for different days and were plotted against time. This electrochemical method based on the voltammetric response showed all the four phases of the growth of the fungus. The growth curve obtained matched well with the conventional methodology, which is obtained by assessing the increase of dry weight of the organisms against time. The electrochemical method is more advantageous than the conventional method because the conventional method is very time consuming and difficult to work with. Further, the electrochemical method clearly shows the decline phase, which is generally not very well defined in the conventional method of assessment of the growth curve. It was confirmed by further experiments that the metabolites were responsible for the anodic peak and not the fungal biomass. Identification of the metabolites that are responsible for the anodic peak is presently being studied.

1 Introduction

The biochemical behaviour of living organisms involves electrochemical relationships. Functions in living cells like the production of ATP, electron transfer in enzymes and subsequent signalling in biochemical processes are a few examples. Numerous biochemical activities and electron behaviour have got very close relationships with life forms and living cells,[1] which makes the study of physiological and biochemical characteristics an important area in bioelectrochemistry. Research areas such as electroporation, direct electron transfer to enzymes and their applications in the areas of cloning, biosensors, *etc.*, are being pursued by a large number of researchers.[2–8] The fast non-invasive measurement of biological cell properties like volume concentration, electrical and morphological parameters is an important area that has applications in monitoring the biomass, sterilization control and the quantitative evaluation of drugs effects.[9–12] In recent years, researchers have developed rapid methods of assessment of cell proliferation.[13,14] There have been reports on methods involving ATP bioluminescence,[15] the antibody-direct epifluorescent filter technique,[16] enzyme immunoassays,[17,18] the polymerase chain reaction,[19] the resonant mirror biosensor[20] and the quartz crystal microbalance biosensor.[21] The passive electrical properties of biological systems were studied by dielectric measurement,[22,23] and there have been reports on electron transfer in protein.[24] Morphological changes of adherent cells have been reported which were studied using electric impedance sensing systems.[25] Various applications of direct voltammetry of cell cultures in the detection of viable microbes in biological samples have been reported.[26–28] Research on the relationships between the population of living cells and the electrode behaviour has been demonstrated.[29]

Recent research has demonstrated that the live microbes *T. shangaiensis*[30] and *S. cerevisiae*[31] could be monitored using cyclic voltammetry (CV). Studies on the acidification of living cells in their environment as the response of cells to a variety of chemical substances have been carried out.[32] However, so far these studies have been confined only to bacterial strains. An attempt has been made here to extend this for fungal strains. The conventional method of the studies on the growth phases of fungi is difficult and time consuming because the time period required for the growth of the fungi is in terms of days, whereas for the bacterial strains all the phases of growth get completed in about a few hours. Here we report the voltammeric behavior of the fungus *Fusarium solani*.

2 Experimental

2.1 Fungal strain, culture media and buffer

The fungus was isolated from soil collected from an industrial site where waste polyurethane scraps were being identified at the Mycology Division, Indian Agricultural Research Institute, New Delhi, India. Czepek–Dox medium containing $KH_2(PO_4)_2$ (1 g), $NaNO_3$ (2 g), $MgSO_4$ (0.5) g, KCl (0.5 g) and glucose (30 g) dissolved in 1 litre of distilled water with pH adjusted to 7.2 was used for the culture. Phosphate buffer (pH 7.2), 100 mM was used for washing the fungal media.

2.2 Electrochemical method and the measurement of the fungal growth in the medium

The measurements were done using a Wenking potentiostat (Tokyo, Japan), Model POS 88, with a Rikadenki (Tokyo, Japan) *X-Y-t* recorder (RW-201 T). The working electrode was polished well before dipping into the fungal broth. All the measurements were made at room temperature (27 °C ± 2 °C). The voltammetric sensing system comprised three electrodes, gold as working electrode, platinum as the counter electrode and calomel as the reference electrode. The fungus *Fusarium solani* was harvested from the whole culture medium following thorough filtration on tarred filter paper after washing it at least thrice with petroleum ether, hexane and methanol.[33] After

† Present address: Institute of BioScience and Technology, Cranfield University at Silsoe, Silsoe, UK MK45 4DT. E-mail: Sri@cranfield.ac.uk

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harvesting, the biomass was dried to constant weight at 95 °C and replicate tests were conducted to obtain average values.

3 Results and discussion

The cyclic voltammogram obtained for the broth containing the fungus at the scan rate of 50 mV s^{-1} has been shown in Fig. 1(a) and (b).

The anodic wave in this experiment appeared at around 0.70 V *versus* SCE. The current of the anodic wave increased with increase in time. The anodic peak that is seen in Fig. 1(a), could be attributed either to the living cell or the extracellular metabolite that is excreted from the fungus during the metabolic activity. A control experiment was carried out as confirmation. The fungus *Fusarium solani* was isolated from the broth by centrifuging and the clear supernatant was collected. The rate and time of centrifugation is as described under Experimental (Section 2.2). The fungus was filtered in a Whatman 40 filter paper and washed thoroughly with phosphate buffer (pH 7.2, 100 mM) to remove any metabolite that adhered to the paper. Centrifugation was again carried out to be sure that the metabolite was devoid of any biomass that could possibly be present in the supernatant. The isolated fungus was suspended in phosphate buffer and the electrochemical characteristics were studied using cyclic voltammetry (CV) [Fig. 1(b)]. It can be seen from Fig. 1 that the CV characteristics are similar to that of the background electrolyte only, indicating that the live cells are not responsible for the anodic wave seen during the growth of the fungus. Similarly, the CV of the supernatant liquid obtained by centrifuging was also recorded. The solution containing the metabolites clearly gave rise to the anodic wave for the microbial broth. To further confirm the result, the absorbance of the metabolite after thorough centrifugation was measured and compared with that of the medium, and was found to be similar. The result suggests that the anodic peak was not from the biomass or from the spores abjected from the conidial head of the fungus but due to the extracellular metabolite. It is very difficult to precisely assign the actual metabolite or group of metabolites that are responsible for the anodic peak. Further work is in progress to down stream the supernatant to identify the metabolite responsible for the anodic wave.

The peak current values were calculated for different days for the fungi studied in this work. The peak current values thus obtained were plotted against the number of days and are presented in Fig. 2. From the plot it was concluded that the electrochemical methodology adopted in this work could clearly show all the four phases of the growth, namely the lag, log stationary and the decline phases, reproducibly, with a relative error of about 7%. It is worth mentioning that earlier authors have reported a similar trend for the microbial species of *Saccharomyces cerevisiae*[30] and *Tetrahymena shangaiensis*.[31] The conventional growth curve could easily be obtained for the bacterial stains, whereas it is more cumbersome in the case of fungi, as colony counting cannot be used. The conventional method still relies on the weight increase during growth of fungi. In addition to the electrochemical experiments another batch was started in parallel, for obtaining the growth characteristics of the fungus by conventional method.

It is well known that in the conventional method, the decline will not be detected clearly for the fungus, as the increase in dry weight is monitored during growth. As seen from Fig. 3, one can observe a constant weight and a marginal weight decrease after the 25 d of incubation. A glance at Fig. 3 clearly shows that the first three phases of the growth match well with the peak current values, reproducibly, with a relative error of about 6%. However, the decline phase obtained by the electrochemical characterization is very well defined in the figure (Fig. 2). This assumes importance for determining the growth characteristics of fungal species for the following reasons. Firstly, the growth time for the fungi is in terms of couple of weeks, when compared to that of bacterial species, which require only about a few hours for the entire growth cycle. By conventional method, the decline phase cannot be estimated properly (Fig. 3). This arises due to the fact that the increase in the weight of the harvested fungus from the broth is used for ascertaining the growth curve. The decay of the dead cells may not take place immediately, thereby rendering it difficult for the conventional method to estimate the decrease in the activity of the living cell, which marks the growth pattern of a fungus. This work assumes importance as the CV data can clearly throw light on the decay phase of the growth pattern of the fungus.

The fungus was scanned for the peak potential as against different scan rates, and as can be seen from Fig. 4, the peak potential is a function of scan rate which, we believe, is one of the characteristic features of a totally irreversible electrode process. It is worthwhile to mention here that this dependence is true regardless of reversibility for any diffusing redox-active species. We also confirmed this effect by studying the linearity

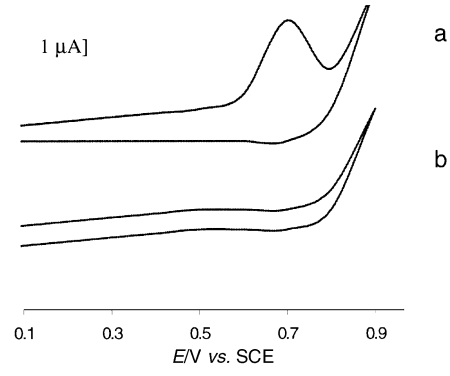

Fig. 1 Cyclic voltammogram of *Fusarium solani* (scan rate of 50 mV s^{-1}). (a) *Aspergillus niger* in broth, 52 mg per 250 ml; (b) fungus in buffer, 52 mg per 250 ml.

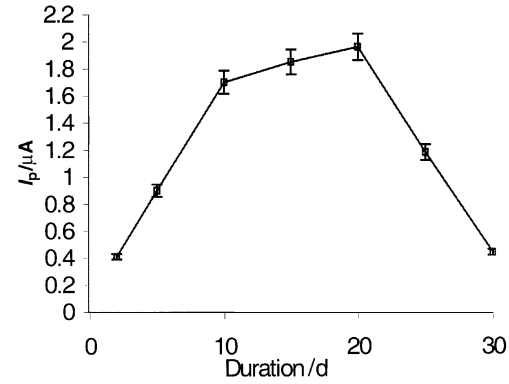

Fig. 2 Growth of *Fusarium solani* in culture media characterised by peak current.

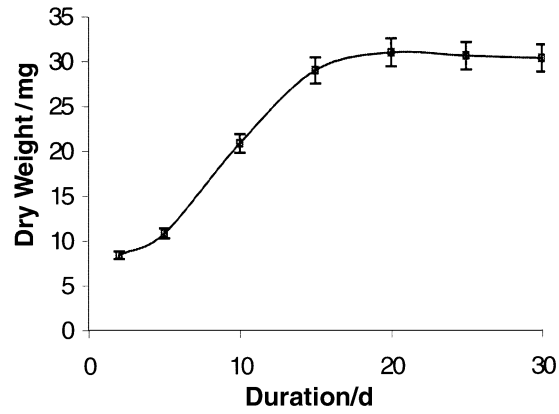

Fig. 3 Growth of *Fusarium solani*, conventional methodology of measurement of dry weight against time.

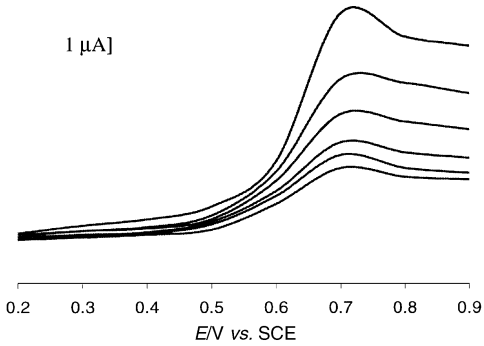

Fig. 4 Voltammetric scan at different scan rates, *Fusarium solani*. Dry wt of 4 mg per 250 ml of media. Scan rates of 5, 10, 20, 50, 100 and 200 mV s^{-1}.

between the square root of the scan rate and peak current. The relationship was found to be linear and was reproducible with relative error of less than 5%. The response is not due to the fungus but due to the electroactive metabolites.

We strongly believe that there could be at least two applications for this technique. Firstly, it could be used for the study of growth phases of organisms which could be an ideal alternative for the complex and time consuming cell counting method. Secondly, the drugs and their effectiveness on the organisms could well be studied using this methodology by finding out the cells' viable state in the culture media. It is very important to bear in mind that the metabolites that are responsible for the generation of the anodic peak need to be identified and the work is still in progress. Once the electroactive metabolites are identified, we believe that areas of research such as biochemical electron transfer in biological systems and studies on effectiveness of drugs on organisms will assume special importance.

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