Short Communication

Voltammetric Studies of *Aspergillus niger*. Assessment of Their Growth Using Electroactive Extracellular Production of Metabolite

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Large number of biochemical activities and electron behavior have a very close relationship with life forms and living cells [1], which makes the study of physiological and biochemical characteristics an important area in analytical chemistry. In recent years, researchers have developed rapid methods of assessment of cell proliferation [2–3], including reports on methods involving ATP bioluminescence [4], antibody-direct epifluorescent filter technique [5], enzyme immunoassays [6, 7], polymerase chain reaction [8], resonant mirror biosensor [9], quartz crystal microbalance biosensor [10], dielectric measurement [11], electron transfer in protein [12], and the electric impedance sensing systems [13]. The applications of direct voltammetry of cell cultures in the detection of viable microbes in biological samples have been reported [14–16]. Recent research has demonstrated that the live microbes, *Tetrahymena shanghaiensis* [17] and the *Saccharomyces cerevisiae* [18], could be monitored using cyclic voltammetry (CV). 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 cumbersome and time consuming because the time period required for the growth of the fungi is in terms of days, while for bacterial strains, all the phases of growth are completed in a few hours. This manuscript discusses the voltammeric behavior of the fungus, *Aspergillus niger* that was isolated from polyurethane waste scraps.

The cyclic voltammogram obtained for the broth containing the fungus at the scan rate of 50 mV/s are shown in Figure 1a and b.

The anodic wave in this experiment appeared around 0.70 (vs. SCE). The current of the anodic wave increased with increase in time. The anodic peak seen in Figure 1a, 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 a confirmation. The fungus, *Aspergillus niger* was isolated from the broth by centrifuging and the clear supernatant was collected. The rate and time of centrifugation is as described under *Experimental*. The fungus *Aspergillus niger*, 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 fungus. Centrifugation was done again 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 voltammogram (CV), (Fig. 1b). It can be seen from the figure that the CV characteristics are similar to that of the background electrolyte only, indicating that the living 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, OD (optical density) of the metabolite after thorough centrifugation was measured and compared

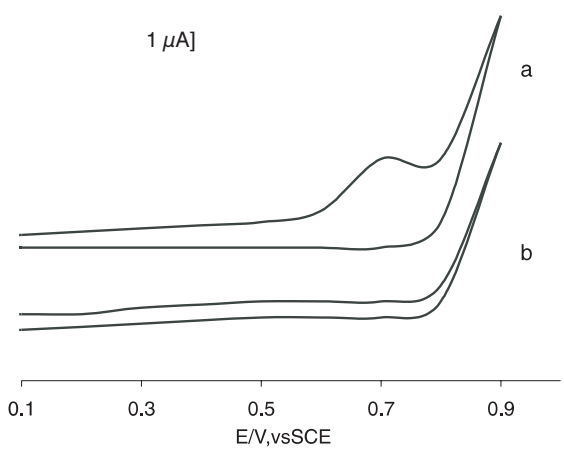

Fig. 1. Cyclic voltammogram of *Aspergillus niger*, scan rate of 50 mV/s, *Aspergillus niger* in broth, 45 mg/250 mL. a) Fungus resuspended in buffer, 45 mg/250 mL.

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with the OD of the media, 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 Figure 2a. From the plot it could be concluded that the electrochemical methodology adopted in this work could clearly show all four phases of the growth, namely the lag, log stationary and the decline phases. The conventional growth curve could easily be obtained for the bacterial stains, whereas it is more cumbersome in the case of fungi, as the colony counting can not 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 Figure 2b, one can observe a constant weight and a marginal weight decrease after 25 days of incubation. A glance of the figure clearly shows that the first three phases of the growth match well with the peak current values. However, the decline phase is very well defined in the figure obtained by the electrochemical characterization (Fig. 2a). This assumes importance for determining the growth characteristics of fungal species for the following reasons. First, the growth time for the fungi is in terms of a 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. 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.

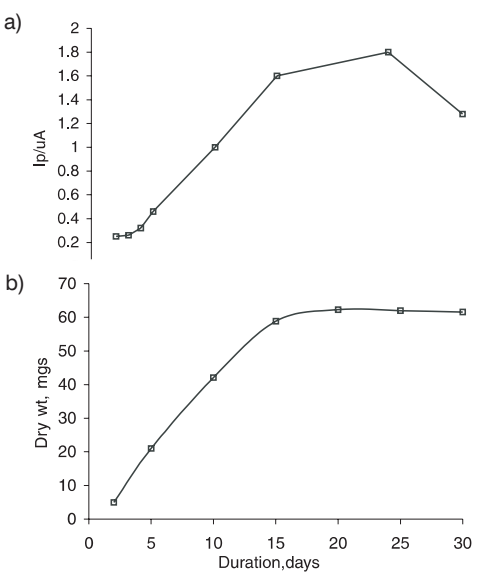

Fig. 2. a) Growth of *Aspergillus niger* in culture media characterized by peak current, reproducible with relative error of about 5 %. b) Growth of *Aspergillus niger*; conventional methodology of measurement of dry weight against time, reproducible with relative error of about 5 %.

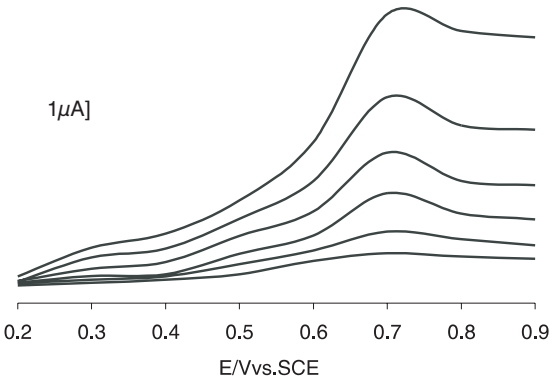

Fig. 3. Voltammetric scan at different scan rates, *Aspergillus niger*, dry wt of 34 mg/250 mL of media. Scan rates of 5, 10, 20, 50, 100, and 200 mV/s.

The fungus was scanned for the peak potential against different scan rates, and as can be seen from Figure 3, the peak potential is a function of the scan rate, which is one of the characteristic features of totally irreversible electrode process. It is worthwhile to mention that this dependence is true regardless of reversibility for any diffusing redox-active species. We also confirmed this effect by studying the linearity 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 % and the coefficient is 0.992. The response is not due to the fungus but due to the electroactive metabolites.

Though it is speculative at the moment to suggest any bioanalytical applications this method could have, we believe that there could be at least the following applications in future. First they could be used for the study of growth phases of organisms which could be an ideal alternative for the cumbersome cell counting method. Moreover 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.

Experimental

The fungus was isolated from soil collected from an industrial site where waste polyurethane scraps were being thrown near Vellore, Tamil Nadu, India. The fungus was identified at the Mycology Division, Indian Agricultural Research Institute, (IARI) New Delhi, India. Czepek-Dox media containing K_2HPO_4 (1 g), $NaNO_3$ (2 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), KCl (0.5 g) and glucose (30 g) were dissolved in one liter 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. The fungal media were autoclaved at 121 °C at 15 psig for 15 min. The fungus was cultured in 500 mL flasks with 100 mL of culture medium. After inoculation the culture was maintained at 27 °C in an aerated condition (in shaker). The isolation of fungus from the

broth was performed, by centrifuging the broth at 3400 rpm for 45 min, at 4 °C.

The electrochemical 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 done at room temperature (27 ± 2 °C). The voltammetric sensing system comprised three electrodes, gold as working electrode (0.2 cm^2), platinum as the counter electrode, and calomel as the reference electrode. The electrode was cleaned well and was cyclically scanned several times from 0.0 to 1.0 V (vs. SCE) for baseline qualification. The fungus was washed well with buffer before experimentation.

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