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Design and Application of an Electrochemical Sensor for Ammonium Monitoring in Bioprocesses

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Introduction

Monitoring and control of manufacturing processes is of great importance in all industries. Bioprocesses use living microorganisms to produce a broad range of valuable goods such as proteins, hormones, enzymes or fermented food products. In a bioprocess, metabolite concentrations vary with the different fermentation phases. Non-ideal mixing conditions further contribute to the formation of local concentration gradients. These conditions render process monitoring a challenging task, specifically in large bioreactors. To ensure high product yields, product quality and process consistency, it is essential to monitor and control the bioreactor, which is tightly connected with the metabolism of the microorganisms. Therefore, being able to measure physico-chemical processes from a quantitative and qualitative perspective is key to achieving successful process control [1]. At many production sites nowadays, simple real-time monitoring systems are already implemented. These systems rely on measurements providing a limited number of variables such as pH, temperature, dissolved oxygen concentration, and carbon dioxide evolution rate (CER). Contrary to that, information concerning biologically important variables such as the concentration of different nutrients, metabolites, and biomass is mainly available by laborious off-line analyses [2]. Over the past decades, there has been an increasing focus on process analytical techniques (PAT) that allow rapid and non-destructive measurements in the frame of fermentation based manufacturing. Therefore, the sensing techniques employed need to be rapid to facilitate this deeper understanding of the process. Rapid quantification is also required for improved process control. On-line monitoring of key primary and secondary metabolites enables fast decision-making through applying dynamic feeding strategies that are tailored to the process conditions. Electrochemical (bio)sensors offer the possibility of on-line monitoring in combination with the advantages of low cost, high sensitivity and selectivity [3].

This work focuses on the development and automation of electrochemical sensors for on-line monitoring of ammonium ions in the fermentation broth. Ammonium is one of the central nutrients in fermentation media and needs to be present at relevant levels to promote growth and enzyme production. Besides, there are also major challenges associated with ammonium. It imposes additional costs on downstream wastewater treatment if more ammonium is added to the medium than is actually consumed by the microorganisms during the fermentation. Usually, the quantification of ammonium in a fermentation broth is performed by spectroscopic methods which are rather time-consuming, considering that a sample preparation is needed in most cases [1]. Microfabricated electrochemical sensors allow for an improved spatial surveillance of production units with sensors placed at several critical locations, and even with the option to incorporate the electrochemical sensors into free-floating sensor particles for data collection in large bioreactors.

Method

An amperometric ammonium sensor is fabricated by immobilization of three different layers on top of a graphene working electrode in a screen-printed electrode (SPE), including electrodeposited copper oxide (Cu_2O), a Nafion membrane and electropolymerized polyaniline (PANi). Prior to use, the electrodes were soaked in phosphate buffer for 30 min at room temperature to equilibrate the sensing composite.

The addition of ammonium ions results in the formation of a complex between Cu(I) and neutral ammonia, which is easily oxidized by dissolved oxygen to Cu(II) with subsequent electrochemical reduction back to Cu(I), observed as a cathodic current increase [4]. Four electron reduction of oxygen down to water illustrates the high reactivity of the Cu(I)-based catalyst. In other words, ammonium complex formation triggers an oxygen reduction reaction (ORR) at the Cu(I)-based species, which in turn, acts to enhance the binding. The whole process is explained in terms of ammonia-associated ORR electrocatalysis, as illustrated in Fig. 1.

Results and Conclusions

Amperometry at -0.45 V was utilized for the measurement of the response to an increase of the ammonium concentration at the PANi-Nafion-Cu-modified electrode in the range of 0.05 to 1.0 mM (Fig. 2). A consistent increase in the cathodic current was observed as a result of the increased ammonium ion concentration. The presence of ammonium causes complex formation with copper, and this causes electroreduction of oxygen to result in an increased current.

The results set the frame for an innovative measuring concept which will be presented. In a traditional bioreactor set-up, data about each measured variable is collected by a single sensor that is mounted in a fixed position close to the reactor wall. In the novel concept, miniaturized biosensors will be mounted on free-floating sensor particles. Data are transmitted by wireless technology as the sensor particle surfaces the fermentation broth. This emerging technology offers great potential for accurate on-line monitoring of fermentation processes. An outlook is given on how novel electrochemical sensors will contribute to monitoring nutrients and metabolites in large scale bioreactors.

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Figure 1. The ORR at the Cu(I)-based PANi composite in the presence of ammonia

Figure 1. Amperometric response of electrochemical sensor to successive additions of NH_4Cl (-0.45 V, phosphate buffer).