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Development of a biosensor for caffeine

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Abstract

We have utilized a microbe, which can degrade caffeine to develop an Amperometric biosensor for determination of caffeine in solutions. Whole cells of *Pseudomonas alcaligenes* MTCC 5264 having the capability to degrade caffeine were immobilized on a cellophane membrane with a molecular weight cut off (MWCO) of 3000–6000 by covalent crosslinking method using glutaraldehyde as the bifunctional crosslinking agent and gelatin as the protein based stabilizing agent (PBSA). The biosensor system was able to detect caffeine in solution over a concentration range of 0.1 to 1 mg mL^{-1}. With read-times as short as 3 min, this caffeine biosensor acts as a rapid analysis system for caffeine in solutions. Interestingly, successful isolation and immobilization of caffeine degrading bacteria for the analysis of caffeine described here was enabled by a novel selection strategy that incorporated isolation of caffeine degrading bacteria capable of utilizing caffeine as the sole source of carbon and nitrogen from soils and induction of caffeine degrading capacity in bacteria for the development of the biosensor. This biosensor is highly specific for caffeine and response to interfering compounds such as theophylline, theobromine, paraxanthine, other methyl xanthines and sugars was found to be negligible.

Although a few biosensing methods for caffeine are reported, they have limitations in application for commercial samples. The development and application of new caffeine detection methods remains an active area of investigation, particularly in food and clinical chemistry. The optimum pH and temperature of measurement were 6.8 and $30 \pm 2\,^{\circ}C$, respectively. Interference in analysis of caffeine due to different substrates was observed but was not considerable. Caffeine content of commercial samples of instant tea and coffee was analyzed by the biosensor and the results compared well with HPLC analysis.

Keywords: Caffeine; Biosensor; Amperometric; Immobilization; Microbial biosensor; High-performance liquid chromatography

1. Introduction

Demand for biosensors has increased markedly in recent years, driven by needs in many commercial and research sectors for specific sensors that are capable of rapid, reliable measurements [1]. Development of biosensors is of interest for diverse applications ranging from biochemical profiling of normal and diseased cells (metabolomics), clinical diagnostics, drug discovery and biodefense, to more straightforward analyses such as fermentation, process monitoring, environmental testing and quality control of foods and beverages. Caffeine (1,3,7-trimethylxanthine) is a natural alkaloid occurring in coffee, cocoa beans, cola nuts and tea leaves. It is mildly stimulating and is used as a therapeutic agent [2]. It is a white compound, moderately soluble in water and organic solvents like ethanol, ethyl acetate, methanol, benzene, etc.

While being a stimulant to the central nervous system, it can have some adverse effects on health. If consumed in excess it can cause adverse mutation effects [3,4]. It is teratogenic, causes inhibition of DNA repair [5], inhibition of cyclic AMP phosphodiesterase activity [5] and inhibits seed germination. It can be a cause of cancer, heart diseases [6] and complications in pregnant women and aging [7].

Coffee is one of the most popular beverages across the world and its caffeine content has an important role in determining the quality of coffee beverages. Further, on account of the harmful effects of caffeine its efficient measurement is relevant. In this context, the development of a sensitive, rapid and cost effective method for monitoring caffeine is greatly needed.

Conventionally, high-performance liquid chromatography (HPLC) separation [8] and UV-spectrophotometric detection [9], methods are applied to both regular and decaffeinated

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green and roasted coffee beans for caffeine content determinations. Other methods such as capillary electrophoresis [10], thin layer chromatography (TLC) [8] and gas chromatography (GC) [11], are used for separation of caffeine in the analysis of mixtures, combined with detection methods such as mass spectroscopy [11] and FTIR spectrophotometry [12]. However, expensive instrumentation, highly skilled technicians and complicated and time-consuming procedures are required for such methods. Another possible technique is flow injection immunoassay using a solid phase reactor, which makes the assay faster because no separation step is needed [13]. However, the time and cost for monoclonal antibody production and purification, and the need for their manipulation with extreme care, are the disadvantages of this approach. A biosensor based on inhibition of 3,5-cyclic phosphodiesterase (CPDE) from bovine heart in combination with a pH electrode for the detection of caffeine in coffee was reported by Pizzariello et al. [14]. The development and application of new caffeine detection methods remains an active area of investigation, particularly in food and clinical chemistry. Significant research and development activity has been devoted to preparing compact analytical devices comprising a bioactive sensing element integrated with a suitable transducing system, known as biosensors, for determination of various inorganic, organic and biological substances. The main advantages of these devices are their specificity, sensitivity and ease of sample preparation, and the fact that no other reagents besides a buffer and a standard are usually required [15]. With these advantages in view, investigations have been carried out in this work to develop a microbial biosensor for the estimation of caffeine in food and beverage samples.

1.1. Principle of microbial based biosensor for the detection of caffeine

Oxidase enzymes utilize molecular oxygen for oxidation of substrate. This oxygen consumption can be monitored when these enzymatic reactions are brought about in the vicinity of a dissolved oxygen electrode. In microorganisms, the enzymatic degradation of caffeine is brought about by sequential demethylation by an oxygenase, into theobromine or paraxanthine. A stoichiometric relation exists between the amount of caffeine converted by the microorganisms and the amount of oxygen consumed based on which, the amount of caffeine in the sample can be determined.

$$\text{Caffeine} + \text{O}_2 \xrightarrow{\text{Enzyme}} \text{Product}$$

2. Materials and methods

Caffeine, theophylline, theobromine, gelatin, glutaraldehyde and para chloro mercuri benzoic acid (Ultra Pure) were obtained from Sigma Chemicals, St. Louis, U.S.A. Analytical grade, dinitro phenyl hydrazine, polyvinyl alcohol and polyvinyl pyrrolidone were procured from Sisco Research Laboratory Chemicals, Mumbai, India. Dehydrated nutrient agar and nutrient broth (AR) were obtained from M/s Himedia Labs, Mumbai, India. All other chemicals were of high purity and were procured from standard sources.

A microbe previously isolated and characterized in our laboratory as *Pseudomonas alcaligenes* MTCC 5264 [16], which was found to have potent caffeine degradation capability was used for the studies. The isolate was cultivated in a modified nutrient broth containing peptone ($1.5\,\text{g}\,\text{L}^{-1}$), beef extract ($5\,\text{g}\,\text{L}^{-1}$), yeast extract ($1.5\,\text{g}\,\text{L}^{-1}$), sodium chloride ($5\,\text{g}\,\text{L}^{-1}$) and caffeine ($0.3\,\text{g}\,\text{L}^{-1}$) adjusted to pH 7.2 to obtain biomass.

2.1. Induction of the organism for caffeine degradation

The caffeine degrading enzyme system has been reported to be highly inducible [17]. The induction was effected by harvesting the biomass and incubating in M-9 medium [18], which was modified by incorporating 0.1% (w/v) caffeine.

The microbial cells accumulated in the nutrient broth were harvested towards the end of logarithmic phase of growth (about 90 h) by centrifugation at $16{,}000 \times g$ and 4 °C for 30 min. The biomass thus obtained as a pellet was washed thrice with cold Phosphate buffer (100 mM), pH 7.2 and aseptically transferred into a 500-mL flask containing 100 mL of caffeine liquid medium (CLM) containing $1\,\text{g}\,\text{L}^{-1}$ of caffeine. This was incubated at 30 °C in an orbital rotary shaker (150-rpm) for a period of 48 h. The cells were then harvested by centrifugation for 30 min at $16{,}000 \times g$ and 4 °C. The pellet of microbial cells was washed 4–5 times with phosphate buffer and stored at 4 °C until further use.

2.2. Construction of the caffeine biosensor

The biosensor comprises the biological sensing element, the transducer, amplification and detector systems as shown in Fig. 1, which was earlier reported [19].

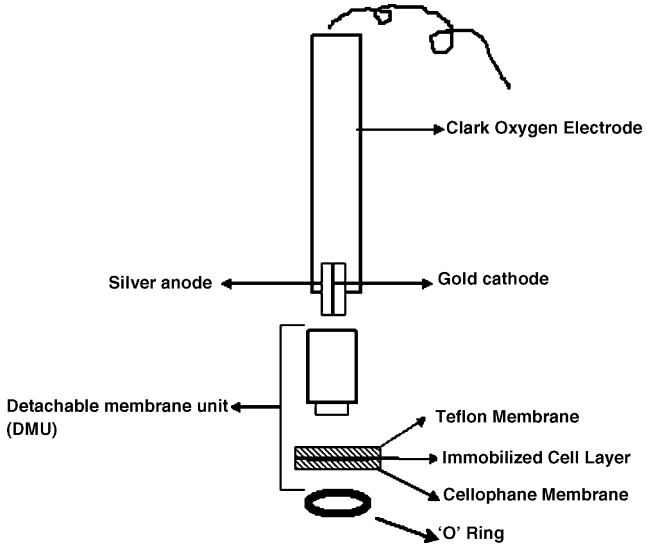

Fig. 1. Schematic diagram of the whole cell electrode used for the biosensor.