Keywords: Biosensor; Cadmium; Milk; Bacillus badius; Urease

Introduction

Milk and dairy products are a source of many valuable nutrients and minerals for human. In recent years, there has been growing interest in microelements, as their presence in food materials is the indicator of qualitative parameters. Trace metals such as Pb, Cd, Zn, Cu, Cr and As are potential bioaccumulative toxins in the production system of milk and dairy products [1]. Cadmium may enter the body through food, water, air or absorption through the skin however food and smoking are the main source of exposure in the non-occupationally exposed population [2]. Cadmium absorption after dietary exposure in humans is relatively low (3–5 %) but cadmium is efficiently retained in the kidney and liver in the human body, with a very long biological half-life ranging from 10 to 30 years. Cadmium is primarily toxic to the kidney, especially to the proximal tubular cells where it accumulates over time and may cause renal dysfunction. Cadmium can also cause bone demineralization, either through direct bone damage or indirectly as a result of renal dysfunction [3].

Lead and cadmium are major contaminant in milk, the statewide survey in California had shown that mean concentration of cadmium in 320 raw milk samples was 6 μg/kg [4]. The average cadmium content in milk from different farm was found to be 0-20 μg/l as reviewed by Valiukenaite et al. [5]. Level of cadmium in the milk of lactating cows reared around lead-zinc smelter and steel manufacturing plant has been recorded as high as 0.23±0.02 μg/ml [6].

Studies suggest that bioavailability of cadmium is different for different food source. Marginal deficiencies of essential nutrients like Zn and Fe enhance the Cd absorption as much as tenfold from the diets containing low Cd concentrations similar to that consumed by populations. As it has been studied that cadmium absorption is increased with an increase of fat and protein content, milk being rich in fat and protein and usually devoid of iron and zinc, is a potential contender for Cd exposure to humans [7,8].

The standard techniques for trace heavy-metal analysis in milk include Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma-Mass Spectrometry (ICPMS). Differential pulse polarographic determination of cadmium, lead and copper in milk has been studied by Tolukoglu et al. [9]. But these methods require expensive equipment which cannot be used in the field. Moreover virtually all of the methods involve complicated and time-consuming sample treatment and pre-concentration steps that can be carried out only by trained professionals. This prohibits screening for heavy metals at various stages of food production and hinders the objective of preventing heavy metal contamination as early as possible in the production chain.

In the recent years application of biosensors for detecting and quantifying heavy metal has been the subject of intense research all over the world [10]. Current study is an effort to develop a disposable optical biosensor for the detection of cadmium in milk.

Material & Methods

All the chemicals and reagent used in the study were of analytical grade. Maya 2000 series spectrometer, fiber optic probe from Ocean Optics has been used in the study.

Preparation of culture

_Bacillus badius_, MTCC 8082, novel isolate of Biosensor Technology Lab, Dept. of Biotechnology, Punjabi University Patiala isolated from urea rich soil near National Fertilizer Limited (NFL), Nangal, India, cultured in the nutrient medium containing 10g Beef Extract, 10g Peptone, 5g Sodium Chloride and 25gUrea/l, pH of the medium was kept 7.0–7.5. Culture was incubated for 21 hrs at 37°C at 200 rpm; average OD of the culture was found to be 0.9 at 600nm of 50 ml broth.

Construction of biocomponent

21 h grown culture of _B. badius_ was centrifuged at 5000 rpm for 20min at 4°C, the pellet of cells from 50 ml culture was taken in 250 μl of 10% glycerol. 50 μl of extracted cells mixed with 600 μl alcohol, 50 μl Tetra Methyl Ortho Silicate (TMOS), 10 μl NaOH (5M) and 20 μl phenol red (1.25mg/ml in 50% Ethanol). 10 μl of the mixture was introduced on to the circular transparent plastic discs with a diameter of 5 mm (Figure 1). The discs thus prepared were incubated for 1hr at 4°C for immobilization. These discs were used for further study.

*Corresponding author: Neelam Verma, Department of Biotechnology, Punjabi University, Patiala, India, Tel: +91-175-3046263; Fax: +91-175- 2286412, 2283073; E-mail: verma.neelam2@gmail.com*

Received October 05, 2010; Accepted October 19, 2010; Published October 20, 2010


Copyright: © 2010 Verma N, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Results and Discussion

A good correlation was found between absorption and the concentration of cadmium in the samples, a linear relation was found between 0.1 μgCd/l to 10 μgCd/l. Conc. of Cd in the milk sample was found to be 8.1 μg/l as calculated from the graph (Figure 3).

Increase in concentration of Cd resulted in the inhibition of urease activity and thereby decrease in production of ammonium ions, decrease in absorption at 555 nm. Though linear relation was found between 0.1 μgCd/l to 10 μgCd/l, convincing results have been shown even at high concentration i.e. 1.0 mg/l, showed more than 90% inhibition (Table 1).

In spiked sample Cd was detected to be 13.32 μg/l for 14.05 μg/l hence reliability of biosensor is found to be as high as 94.8% (Table 2). Biocomponent was found to be stable for more than 90 days when stored at 4°C in 10% glycerol.

Biosensors for heavy metal, especially for Cd reported earlier includes Tauriainen et al. [11] that constructed a luminescent bacterial biosensor respond to cadmium at 3.3 n mol/l; the results are obtained with 2-3 hrs incubation. Biosensor developed in present study is rapid as well as sensitive to a lower concentration. May and Russell [12] developed a biosensor based on changes in structure of urease enzyme after binding with cadmium being the basis of surface plasmon resonance biosensing system. The enzyme was modified with N-succinimidyl 3-(2-pyridylthiol) propionate (SPDP) to facilitate the formation of a self assembled monolayer of urease on the gold coated glass SPR sensor disk. It is this change of enzyme monolayer measured by SPR, which has been related to the cadmium ion concentration in the range of 0-10 mg/l. Current study is novel and significant for its source of enzyme i.e. urease, bio-sensing system with a quick response time; having a significantly low detection limit (0.1 μg/l). A biosensor based on mammalian metallothionein for the detection of metal ions was developed by Wu and Lin [13], that detected the cadmium up to the range 2 to 10 μmol/l. Low cost small size all-solid state pH-urease electrodes useful for determination of heavy metals ions have been developed by means of screen printing. Cadmium was inhibiting at the conc. of 1 mM/l [14]. Our developed biosensor is much more sensitive.

A conductometric biosensor using immobilized Chlorella vulgaris microalgae was used as a bienzymatic biosensor with limit of detection 10 μg/l for Cd after 30 min long exposure based on alkaline phosphatase, acetyl choline transferase inhibition [15]. Our developed biosensor is faster as well as sensitive to a lower concentration. Liao et al. [16] developed a GFP based biosensor that responded to Cadmium 0.1 n mol/l being the lowest detectable concentration with 2 hr exposure. Haron and Ray [17] developed an optical biosensor for cadmium and lead by employing the technique of total reflection at the interface between Si3N4 core and composite polyelectrolyte self-assembled (PESA) membrane containing cyclopettachromotropylene (CTCT) as an indicator; achieved a detection limit as low as 1 μg/l for both the metals. Chong et al. [18]
developed a whole cell biosensor on a diamond electrode. Unicellular microalgae *Chlorella vulgaris* was entrapped in the BSA membrane and immobilized directly onto the surface of a diamond electrode for heavy metal detection. The cell based diamond biosensor could attain a detection limit of 0.1 μg/l for cadmium. Application of developed biosensor has not been highlighted.

A novel transmission-based localized surface plasmon response (LSPR) fiber-optic probe has been developed to determine Cd ion concentration with a detection limit of 0.16 μg/l though the application. The sensor retained 85% of its original activity after nine cycles of deactivation and reactivations; in addition sensor retains its activity up to 35 d at 4°C in 5% d-(+)-trehalose [19]. Application of the developed biosensor has not been highlighted. In comparison our developed biosensor is more stable.

In comparison of biosensors developed earlier present biosensor has got many advantages like lower detection limit, quick response, storage stability, miniaturization of volume to a level of 10μl never achieved before. The basis of fastness and sensitivity may be the novelty of the biocomponent as it all depends on the orientation of enzyme, structure and the quantity [20,21].

## Conclusion

Conclusively the study resulted in the development of a fast and reliable absorption-transmission based fiber optic biosensor with a lower detection limit for cadmium equivalent in milk. Further specificity can be achieved by passing the milk through cadmium specific column.

## Acknowledgement

The authors are thankful to the ICAR (NAIP), New Delhi, India for the research grant C4/C10125/2008 supporting this work.

## References