Development of Optical Microbial Biosensor for Reflectometric Nitrite Ion Detection

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Abstract

In this paper, we have discussed a microspheres-based microbial optosensor for NO_2^- ion quantitation was constructed by using immobilized *Raoutella planticola* (*R. planticola*), the bacterium expressing NAD(P)H nitrite reductase (NiR) enzyme, which was isolated from local edible bird's nest (EBN) *via* microbial technique. The whole cells and the lipophilic Nile Blue chromoionophore (NBC) were physically adsorbed on the self-adhesive photocurable poly(*n*-butyl acrylate-co-*N*-acryloxysuccinimide) [poly(nBA-NAS)] microspheres, whilst the reduced co-enzyme NAD(P)H was covalently immobilized on the succinimide-functionalized acrylic microspheres *via* peptide link to produce a reagentless nitrite biosensing system. As the microbial biosensor responded to nitrite through color change from blue to pink, a facile reflectometric approach was adopted to measure the reflectance intensity at 639 nm, before and after reaction with nitrite at optimum pH 8. The optosensor could quantify NO_2^- ion concentration within a dynamic linear response range of 0.5-400 mg L^{-1} with a limit of detection (LOD) of 0.2 mg L^{-1}. The large surface area to volume ratio of the acrylic microspheres allowed solid-state diffusional mass transfer of the substrate to occur at the micro-biosensor surface, and an equilibrium response was achieved within 5 min. The reflectometric microbial biosensor exhibited high specificity to NO_2^- ion with negligible response to some other nutritionally important minerals (i.e. NH_4^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Fe^{2+} and NO_3^- ions), which may co-exist with the target NO_2^- ion in food, water and environmental samples. The practical feasibility of using the bio-optode for nitrite assay in food matrix sample showed good agreement with the standard ion chromatography method.