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8-hydroxyquinoline-glucuronide sodium salt used as electroactive substrate for a sensitive voltammetric detection of *Escherichia coli* in water samples

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Abstract

Escherichia coli are fecal indicator organisms and can represent a serious health hazard. The aim of our study was the development of a new and simple electrochemical method for a fast and sensitive detection of *E. coli*. Therefore, the β-D-glucuronidase (GUS) enzyme production was stimulated. The GUS activity promoted the cleavage of a specific substrate into an electroactive compound that was oxidized using a potentiostat leading to a signal in a specific current output range. The principle of the assay was tested with enzyme solutions before living cells were investigated. The output signal was continuously measured, indicated enzyme activity and subsequently, was an evidence for *E. coli* cells. After verification of the methodology, we were able to specifically detect one colony-forming unit per ml within 10 h. The developed method comprises of only a few working steps and allowed a sensitive identification without any interference from other bacteria.

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Keywords: E. coli; voltammetric detection, 8-hydroxyquinoline glucuronide; electrochemistry

1. Introduction and Methodology

Escherichia coli are the most prominent representatives of our bacterial gut flora. Some serotypes can cause serious diseases and therefore, the identification of fecal contaminations with *E. coli* is essential. Nowadays, the classical cultivation takes up to 3 days to identify this indicator organism.

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The aim of our study was the development of a new electrochemical method for a fast and sensitive detection of *E. coli*. In this assay the GUS enzyme production was induced by adding methyl-β-D-glucuronide sodium salt (MetGlu). The GUS activity promoted the cleavage of 8-hydroxyquinoline glucuronide (8-HQG) or 8-hydroxyquinoline glucuronide sodium salt (8-HQG-SS), respectively, to the electroactive compound 8-hydroxyquinoline (8-HQ) [1]. This substance could be oxidized on the working electrode of a potentiostat using cyclic voltammetry leading to a specific current output signal [2, 3]. The potentiostat used for all measurements was developed at our group [4]. The principle of the assay was tested with different enzyme solutions of GUS before *E. coli* cells were investigated [5] (Fig. 1). The output current signal was continuously measured (up to 10 h), indicated enzyme activity and subsequently, was an evidence for *E. coli* cells. Figure 1 gives a general overview of the experimental steps of the designed assay. For evaluation of the optimal incubation temperature *E. coli* and *Citrobacter freundii*, both in different concentrations were tested at 37 °C and 44.5 °C, respectively. Furthermore, different dilutions of *E. coli* overnight cultures were investigated in order to define the detection limits of the method. Additionally, various bacterial strains were analyzed for cross-reactivity and false-positive results, respectively. Finally, the developed methodology was applied for filtered water samples.

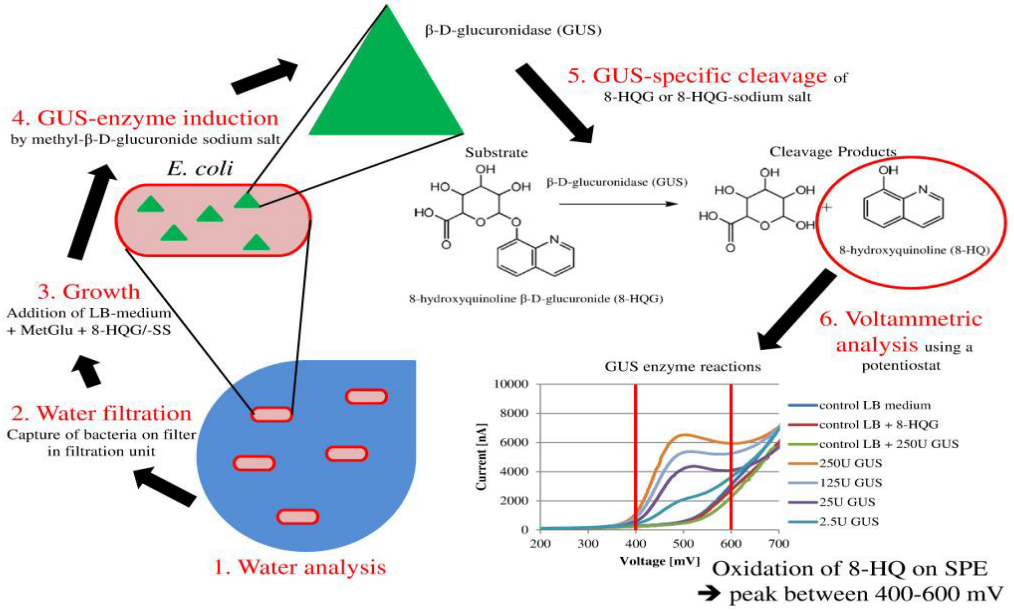

Fig. 1. General overview of the experimental setup. *E. coli* bacteria are captured on a filter membrane and incubated with LB medium supplemented with MetGlu as inducer for the GUS enzyme production and with 8-HQG or 8-HQG sodium salt as substrate for the GUS-enzyme. The electroactive cleavage product 8-HQ can be oxidized on screen printed electrodes (SPE) resulting in a specific current output signal in the range between 400-600 mV. Results of GUS enzyme solutions (2.5-250 U/ml) are shown after 15 min incubation.

2. Results and Discussion

The voltammograms of the different enzyme solutions and of the bacterial cultures demonstrated the typical s-shape signal with a peak between 400 to 600 mV (Fig. 1 and 2a) [1]. In Figures 2a and 2b the results for the evaluation of the optimal incubation temperature are shown. *E. coli* bacteria favoured 44.5 °C for their growth, enzyme production and –activity, whereas *C. freundii* preferred 37 °C for growing in LB medium.