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Novel sensor platform for rapid detection and quantification of coliforms on food contact surfaces

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ABSTRACT

In this paper, a novel sensor platform based on screen printed carbon electrode coated by graphene modified polyacrylamide gel (GR/PAAGC) was developed and implemented for sampling, detection and enumeration of coliform bacteria (coliforms) on food contact surfaces. The optimized formula of polyacrylamide (PAA) and agar-agar increased the adhesive properties of the gel, being crucial for the coliforms recovery, attached to food contact surfaces. The 6-Chloro-3-indoxyl-β-D-galactopyranoside (6-CIGP) was used as a new electrochemical reporter for β-D-galactosidase activity. The released 6,6′-Dichloro-Indigo (6-DI) was directly detected by GR/PAAGC sensor. The presence of Isopropyl-β-D-thiogalactopyranoside (IPTG) and n-Octyl-β-D-thiogalactopyranoside (OBDG) in the gel contributed to reduction of the detection time. The addition of graphene enhanced the voltammetric signal and increased the conductivity of PAA gel. The anodic and cathodic peaks of the released product were directly proportional to the concentration of coliforms. Bacterial cell concentrations ranging from $1.6\log_{10}$CFU/mL to $6.6\log_{10}$CFU/mL were detected. Well-shaped, sharp voltammetric curves were generated within 3 h. Redox peaks exhibited good sensitivity with detection limits (LOD) $< 0.6\log_{10}$CFU/mL. After series of optimization experiments, coliforms ranging from $0.6\log_{10}$CFU/cm^2 to 6.6_{10}CFU/cm^2 on stainless steel surfaces have been detected within 30 min with a LOD of $0.1\log_{10}$CFU/cm^2. The developed rapid, sensitive, reproducible and specific sensor successfully applied for single detection as well as for real-time monitoring of growth of coliform bacteria on stainless steel surfaces during food processing.

1. Introduction

The microbiological data, obtained within the food processing plant during different periods, play key role in proper verification of food management programs (Milios et al., 2014). Detection and control of indicator and pathogenic microorganisms are another important steps for implementation of Good Hygienic and Good Manufacturing Practices (Petruzzelli et al., 2018).

Indicator microorganisms have been defined as a group of microorganisms used to show general poor hygienic conditions where there is an increased risk for food to be contaminated by pathogenic bacteria (Buchanan and Oni, 2012; Ceuppens et al., 2015). Bacterial groups including coliforms (Enterobacterales with expression of beta-galactosidase), *Enterobacterales*, and total gram-negative organisms represent indicators of poor sanitation or post processing contamination in different food processing industries (Biasino et al., 2018; Hervert et al., 2017; Kundu et al., 2018; Schill et al., 2017). *Escherichia coli (E. coli)* was identified as a useful indicator organism to verify the adequacy of the food safety management programs in bovine, swine and poultry slaughterhouses (Belluco et al., 2016).

In food processing factories contact surfaces act as reservoirs of bacteria and one of the main sources of contamination and cross contamination of food at different production stages. The role of contaminated surfaces in spreading pathogenic and indicator bacteria such as *Salmonella spp.*, *E.coli* and coliforms to foods is already well established in food processing, catering, and domestic environments (Falcó et al., 2018; Saad et al., 2013; Valero et al., 2017). The pathogens are often found attached on the stainless steel food-contact surfaces of processing equipment, knives, cutting boards and the conveyor belts. Mentioned microorganisms can contaminate the finished products (after processing) via direct- and/or indirect-contact of the food and contaminated surfaces of processing environments, including the surfaces that are distant from the products, such as the floor and walls in the processing areas (Keeratipibul et al., 2017; Piovezan et al., 2014).

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Another important aspect of attachment of microorganisms to stainless steel surfaces is the formation of biofilms. Bacterial cells are normally released from biofilms, causing discontinuous secondary contamination of foods during their processing (Buzón-Durán et al., 2017; Szczepanski and Lipski, 2014). Therefore, it is crucial to detect bacteria on food contact surfaces before formation of biofilms as well as to detect target bacteria in complex biofilm. Thus, for food processing industry it is essential to count with an early warning sampling and detection method: for real time monitoring of food contact surfaces contamination, in time prevention of cross contamination and in place assessment of efficiency of cleaning and disinfecting procedures.

In recent years, electrochemical detection of foodborne pathogenic and indicator bacteria based on their specific enzyme activities has been intensively studied to develop highly sensitive, small and simple biosensors (Adkins et al., 2017; Chen et al., 2015; Manibalan et al., 2015). The most of the developed electrochemical techniques have been designed for detection of target bacteria in water samples with relatively easy filtration method (where the filtration used as immobilization of bacteria on substrate) comparing to challenging sampling of bacteria from food contact surfaces. To our knowledge, there is no available low cost, single-step electrochemical device/sensor designed for real time monitoring of growth, detection and quantification of coliforms on food contact surfaces in food processing industry during production shift.

Possession of the gene *lacZ*, is the most prominent feature of the coliforms (Molina et al., 2015). In coliforms the lacZ gene product β-D-galactosidase is responsible for cleavage of lactose into glucose and galactose. Traditionally, total coliforms have been defined based on their ability to ferment lactose to acid and gas. With the introduction of enzyme-based tests, the definition includes bacteria within the family Enterobacteriaceae that possess the β-D-galactosidase enzyme (Zhang et al., 2015). β-d-galactosidase-positive reaction has been included also in to the definition of coliform bacteria by APHA standard (APHA, 2005). Currently, the coliform bacteria are located in several families which constitute the recently described order *Enterobacterales* (Adeolu et al., 2016).

To date, the chromogenic indoxyl based substrates have been used only for optical detection of β-galactosidase to identify and quantify the coliforms (Gunda et al., 2017; Orenga et al., 2009). The electrochemical detection of coliform bacteria using 6-Chloro-3-indoxyl-β-D-galactopyranoside substrate has never been reported. Thus, the aim of this study was to design the platform for novel electrochemically active gel modified sensor for the specific and rapid detection and quantification of Coliforms/E.coli on food contact surfaces.

2. Materials and methods

2.1. Bacteria cultivation and enumeration

Freeze-dried cultures of *E.coli* DSM 498, *E.coli* DSM 301, *Hafnia alvei* DSM 30163^T, *Klebsiella pneumonia* DSM 30104, *Bacillus pumilus* DSM 27 ^{T} and *Pseudomonas fluorescens* DSM 50090 ^{T} were obtained from Leibniz Institute DSMZ-German Culture Collection. Each strain was revived by adding 0.5 mL of liquid media to the dry pellet. Suspended cultures were transferred to Nutrient broth and Nutrient agar plates (Sigma Aldrich®, Germany) and were incubated at 37 °C (*H. alvei* DSM 30163^T, *B. pumilus* DSM 27 ^{T} *and P. fluorescens* DSM 50090 ^{T} at 30 °C) for 24 h. The coliform strains *E.coli* DSM 498, *E.coli* DSM 301, *H. alvei* DSM 30163^T, and *K. pneumonia* DSM 30104 were cultured on HiCrome™ Coliform agar (Sigma Aldrich, Germany) in order to confirm their β-galactosidase activity and to increase strain selectivity. Cocktail of coliform bacteria where prepared by transferring of each coliform strain (half of 3 mm loop) to 200 mL nutrient broth. The viable concentration of the mixed coliform culture was determined by monitoring of the growth by optical density (OD_{600}) and plate count method during 8 h by shaking at 37 °C. For each new experiment, the same inoculation conditions were used. The working suspension with the inoculation level of $4x10^8$CFU/mL was centrifuged at 7000 rpm/min for 20 min, washed twice with phosphate buffer saline (PBS, pH 7.4) and re-suspended in physiological saline solution. Suspended cultures were serially diluted and enumerated to verify the initial concentration. Coliforms culture was further diluted to reach concentrations ranging from $0.6\log_{10}$CFU/mL to $8.6\log_{10}$CFU/mL to use them in the following experiments.

2.2. Bacterial attachment and enumeration on stainless steel surfaces

Stainless steel coupons (5cmx5cm, Type 304, 2b finish) were cleaned using acetone and alkaline detergent. The cleaned coupons were boiled in distilled water (DW) for 15 min, dried for 24 h, and autoclaved at 121 °C for 15 min as described by (Son et al., 2016). After this period, the center of each 25cm^2 coupon was inoculated with 0.5 mL of suspension containing coliform cultures with concentrations ranging from $0.6\log_{10}$CFU/mL to $8.6\log_{10}$CFU/mL. To simulate working and dirty conditions, meat juice was used as soiling agent. Meat juice was prepared by homogenization of fresh minced meat and further filtration with paper filter. Filtered juice was sterilized by syringe using 0.2 μm, sterile, PTFE membrane filters (Millipore, Germany). Sterile meat juice was stored at -20 °C for future experiments. The meat juice was transferred, spread and dried (for 30 min) on the surface before inoculation of the surface with the coliform bacteria. Verification of final concentration of the attached coliform bacteria on the clean (sterile DW) and soiled (meat juice) stainless steel surfaces was done by wet-swabbing and agar touching methods according to (ISO18593, 2004) and (FNES4, 2017) standards.

2.3. Testing of bacterial attachment to gels

Four types of gels containing 5 g/L Peptone, 5 g/L NaCl, 2 g/L KH_2PO_4, 2 g/L K_2HPO_4, 10 g/L agar-agar, 1 g/L polyacrylamide and 3 g/L meat extract (Carl Roth, Germany) in different combinations have been tested. After sterilizing at 120 °C for 15 min., 20 mL of each gel was uniformly poured into plastic petri dishes and stored at 4 °C for 18 h. Stainless steel coupons were inoculated at different concentrations of coliform bacteria ($0.6\log_{10}$CFU/cm^2 to $3.6\log_{10}$CFU/cm^2) by the above-described method. After drying, the stainless steel coupons were attached to the surfaces of four different gels for 5 min. To ensure an equal pressure and attachment conditions for every sample, 300 g weights were placed on top of each coupon. After incubation at 37 °C for 24 h, the attached colony form units were counted.

2.4. Optimization of pH of the gel

The gel, with the highest attachment efficiency, was used for testing the influence of pH on galactosidase activity. The gels with the pH values ranging from 6.0 to 8.0 were prepared by addition of 1 M HCL or 0.1 M NaOH, respectively. After sterilization, the gels were cooled down to 50 °C following with addition of 0.5 g/L 6-CIGP and 0.25 g/L IPTG (Carl Roth, Germany). After appropriate mixing, the gels were poured into 6-well polystyrene plates (VWR, Germany) and dried for 2 h. Then, 0.01 mg/mL β-D-Galactosidase (Sigma-Aldrich, Germany) diluted in PBS was injected to all gels, except the blanks. Thereafter, part of the plates were placed in to microplate reader (Synergy MX, Biotech, Germany) for detection of optical signal and the other part were placed in the incubator for visual control of color formation. The plates were incubated at 42 °C for 2 h period. Absorbance was measured at 530 nm wavelength in visible spectrum.

2.5. Preparation of graphene solution

Hundred microgram of hydrophilic graphene nanoplatelets (Sigma-Aldrich, Germany) were dispersed in 1:1 mixture of DMF (*N*,*N*-