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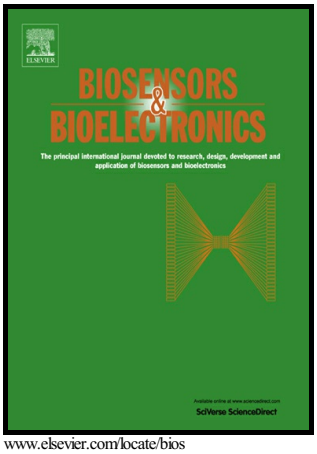

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Whole cell based microcontact imprinted capacitive biosensor for the detection of *Escherichia coli*

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Abstract

In this study, a label-free, selective and sensitive microcontact imprinted capacitive biosensor was developed for the detection of *Escherichia coli*. The recognition of *E. coli* was successfully performed by this sensor prepared with the combination of microcontact imprinting method and capacitive biosensor technology. After preparation of bacterial stamps, microcontact- *E. coli* imprinted gold electrodes were generated using an amino acid based recognition element, N-methacryloyl-L-histidine methylester (MAH), 2-Hydroxyethyl methacrylate (HEMA) as monomers and ethyleneglycol dimethacrylate (EGDMA) as crosslinker under UV-polymerization. Real-time *E. coli* detection experiments were carried out within the range of 1.0×10^2–1.0×10^7 CFU/mL. The unique combination of these two techniques provides selective detection with a detection limit of 70 CFU/mL. The designed capacitive sensor has high selectivity and was able to distinguish *E. coli* when present together with competing bacterial strains which are known to have similar shape. In addition, the prepared sensor has the ability to detect *E. coli* with a recovery of 81-97% in e.g. river water.

Keywords: Microcontact imprinting, capacitive biosensor, N-methacryloyl-L-histidine methylester, *Escherichia coli*

1. Introduction

Escherichia coli is a common and nonpathogenic member of the organisms which colonize the gastrointestinal flora of healthy humans and animals. Most strains of the organism are harmless, but some of them are pathogenic. *Escherichia coli* (O157:H7) is a predominant enteropathogen which may cause bloody diarrhea, kidney failure and central nervous system complications (Nataro and Kaper, 1998). It has a very low dose for infecting human, only as few as ten cells may be sufficient to cause infection (Cornick and Helgerson, 2004; Roy et al., 2015).

Rapid and early detection of the causative agents leading to serious diseases and lethality is a great concern, due to increased frequency of microbial contamination of food and watersupplies (Roy et al., 2015). Current and conventional identification methods of these microorganisms are time-consuming, laborious and expensive (Ahmed et al., 2014; Roy et al., 2015). Therefore, there is a need for more rapid and cost-effective analytical techniques for microbial detection (Ahmed et al., 2014).

Biosensor technology has been introduced as a promising tool for microbial detection in various applications such as clinical diagnosis, water and food analysis, bioprocess and environmental monitoring (Ahmed et al., 2014; Singh et al., 2014). Biosensors often show high selectivity (Lu,et al, 2014; Leung et al., 2015) and sensitivity (Zhang et al., 2015) of specific biological analytes in complex media (blood, serum, urine, food, water, etc.) with minimum sample pre-treatment (Ahmed et al., 2014). In recent years, many attempts have been made to develop sensors for detection of whole bacterial cells by using impedimetric and optical sensors (Ahmed et al., 2014). Among electrochemical biosensors, capacitive biosensors have attractive properties such as label-free, real-time and rapid detection of macro- and small molecules along with both high selectivity and low detection limit (Lebogang et al., 2014; Mahadhy et al., 2014). The capacitance measurement principle of these sensors relies on the changes in dielectric properties of the electrode surface. The interaction between analyte of interest and electrode surface leads to a decrease in capacitance (Berggren et al., 2001; Gutierrez et al., 2015).

Molecular imprinting is a well suited technique serving the purpose to develop highly selective sensing devices. Attention has been directed to the combination of molecular imprinting with biosensor technology for the detection of microorganisms (Findeisen et al., 2012; Roy et al., 2015; Yilmaz et al., 2015). Use of molecular imprinting method makes it possible to create specific recognition cavities which are similar in size, shape and/or have a matching chemical functionality to that of the template (Iskierko et al., 2016). Microcontact imprinting is based on the polymerization of a thin layer of monomers when a target stamp has been placed on the surface of the sensor prior to polymerization. When the stamp with its immobilized target molecules or cells is removed, it leaves behind a polymer surface with cavities matching the shape of the targets on the stamp (Sener et al., 2013). One unique feature is that the print structure is never fully embedded in the polymer, thereby making it easier to remove the template and to rebind macromolecular and even particulate structures (Ertürk and Mattiasson, 2015; Irudayaraj et al., 2012).

Several studies have been reported on sensor applications of microcontact molecular imprinting in order to detect a great variety of target molecules, including proteins (Ertürk et al., 2014; Ertürk et al., 2015) and hormones (Sener et al., 2013). However, there have been only a few studies focused on whole cell imprinting (Cohen et al., 2010; Roy et al., 2015; Yilmaz et al., 2015). Recent methodological advances in the field of whole-cell detection involve the improvement of cell complementary surfaces in which the cells were used as templating molecules using molecular imprinting technique along with biosensor technology (Dickert and Hayden, 2002; Tokonami et al., 2013; Bole and Manesiotis, 2015). There is a need to achieve low detection limits with high selectivity and sensitivity in order to quantify microorganisms on molecularly imprinted polymer sensors.

Microcontact imprinting technology was combined with capacitive biosensor technology for the detection of a selected model bacteria: *E. coli*. The prepared electrodes were characterized by cyclic voltammetry (CV), atomic force microscopy (AFM) and scanning electron microscopy (SEM). After real-time detection experiments, selectivity of the *E. coli*-imprinted

electrode was tested with other bacterial strains such as *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), and *Salmonella paratyphi* (*S. paratyphi*). The efficiency of the MIPs was shown by comparing the results obtained with those from a non-imprinted (NIP) electrode. Experiments were also carried out using river water and apple juice samples.

2. Materials and Methods

2.1. Materials

E. coli ATCC 25922, *S. aureus* ATCC 25923, *B. subtilis* ATCC 23857, *S. paratyphi* ATCC 9150 were obtained from the Culture Collection Laboratory of Department of Biology (Biotechnology), Hacettepe University, Turkey. Tyramine (99%, $HOC_6H_4CH_2CH_2NH_2$), lysozyme (from chicken egg white), 2-hydroxyethyl methacrylate (HEMA) and ethyleneglycol dimethacrylate (EGDMA) were supplied from Sigma Chemical Co. (St. Louis, USA). Acryloyl chloride and 1-dodecanethiol were from Aldrich (Deisenhofen, Germany). Glutaraldehyde 50% (w/v), triethylamine, 3-amino-propyl-triethoxysilane (APTES) and α-α'-azoisobutyronitrile (AIBN) were from Fluka (Buchs, Switzerland). N-methacryloyl L-histidine methylester (MAH) was supplied from Nanoreg (Ankara, Turkey). Luria Bertani (LB) broth and Gram Staining Kit were purchased from Sigma–Aldrich Chemie, Switzerland. Glass slides (26 × 76 mm) (Menzel-Glaser) were used as the base for bacteria stamp in microcontact imprinting. All other chemicals used were of analytical grade. All buffers were prepared with water processed using a reverse osmosis step with a Milli-Q system from Millipore (Bedford, MA, USA). Prior to use, all buffers were filtered through a Millipore filter (pore size 0.22 μm) and degassed for 1 h.

2.2. Bacteria sample preparation

E. coli, *S. aureus*, *B. subtilis*, *S. paratyphi* strains were used in the experiments. The bacterial strains were inoculated into LB broth (100 mL in 250 mL Erlenmeyer flask) and incubated at 37°C for 18h with constant shaking at 150 rpm. The viable counts were determined by serial 10-fold dilutions in sterile 10 mM PBS (phosphate buffered saline) buffer and 0.1 mL of aliquots of each dilution was plated onto LB agar plates in triplicate. The plates were incubated overnight at 37°C and the colonies on the plate were counted. The concentration of bacteria was calculated in colony forming units per milliliter (CFU/mL). After incubation, one milliliter aliquot of each bacterial culture was centrifuged at 6000 g for 15 min at 25°C and the culture supernatant was discarded. Then, the bacterial pellets were resuspended in 1 mL sterile PBS and centrifuged at 6000 g for three times and with Tween 80 (0.2%) for two times in order to disperse aggregated cell. The concentrated pellet was resuspended in 1 mL sterile distilled water.

2.3. Preparation of the microcontact-*E. coli* imprinted electrodes

2.3.1. Preparation of the glass slides

Glass slides (26 × 76 mm) were cleaned in three steps using 10 mL of 1 M HCl, 1 M NaOH and ethanol, respectively in ultrasonic cleaner for 10 min in each step. They were rinsed with

de-ionized water between each step and then, dried with nitrogen gas. The cleaned slides were modified by reacting with 10% APTES in ethanol (v/v) at room temperature for 1 h in order to introduce amino groups to the surface. Then, they were rinsed with ethanol to remove noncovalently bound APTES and dried with nitrogen gas. The APTES-modified surface was immersed into 5% (v/v) glutaraldehyde solution in 10 mM sodium phosphate buffer (pH 7.4) at room temperature for 2 h to obtain aldehyde groups on the surface. Then, the glass slides were rinsed with sodium phosphate buffer for removal of unbound glutaraldehyde. They were washed with distilled water and dried with nitrogen gas (Ertürk et al., 2014). For immobilization of *E. coli* (0.5×10^8 CFU/mL), 200 µl of *E. coli* suspension (in deionized water, pH 7.4) was dropped onto the glass slides and dried at 25°C overnight for the immobilizing the cells onto the surface. Finally, the glass slides were washed with deionized water and dried with nitrogen gas. They were kept at 4°C in a closed Petri dish until use.

2.3.2. Preparation of the capacitive gold electrodes

The electrodes consisted of a sheet silica with a deposited thin layer of chromium and of top of that a 200 nm thick layer of gold. The shape of the gold electrode was circular and with a diameter of 3 mm.

Firstly, the gold surfaces of electrodes were cleaned with ethanol, de-ionized water, acetone, de-ionized water and Piranha solution (3:1, concentrated H_2SO_4: 30% H_2O_2, v/v), de-ionized water, respectively for 10 min in each step in ultrasonic cleaner and dried with nitrogen gas. Following these, the electrodes were cleaned in plasma cleaner (Mod. PDC-3XG, Harrick, NY) for 30 min. For the introduction of free amino groups on the gold surface of the electrode, electropolymerization of tyramine was implemented in ethanolic solution of 10 mM tyramine in the ratio of 3:1 by cyclic voltammetry (CV), applying potential sweeps between 0–1.5 V (vs. Ag/AgCl) and a scan rate of 50 mV s^{-1} for 15 cycles (Teeparuksapun et al., 2010). After rinsing with de-ionized water, the coated electrodes were dried with nitrogen gas. Secondly, the electrodes were immersed in a solution containing 30 mM acryloyl chloride and 30 mM triethylamine (in toluene) overnight at room temperature. Acryloyl chloride formed amide groups with the amino groups on the surface of the electrode and left behind vinyl groups that could take part in the subsequent polymerization process. After rinsing with de-ionized water the electrodes were dried with nitrogen gas. The degree of insulation of the electrode surface after each step was measured using CV (Ertürk et al., 2014).

2.3.3. Microcontact imprinting of *E. coli* onto the capacitive gold electrode

In the first step, the mixture of MAH, $Cu(NO_3)_2.2.5H_2O$ (0.2 mg) and de-ionized water was mixed for 1 h for preorganization. For the polymerization, the reaction mixture consisting of HEMA (13 µL) as monomer, EGDMA (40 µL) as cross linker, MAH-Cu(II) complex as pre-organized monomer were mixed for 5 min. Then, the initiator 0.1 mg AIBN was added into this stock solution. 1.5 µL was taken from this reaction mixture and dropped onto the surface of the premodified gold electrode. Then, *E. coli* cells immobilized on a glass slide were

brought into contact with this monomer mixture covering the electrode surface. The polymerization was initiated under UV light (100 W, 365 nm) and proceeded for 20 min. After the removal of the stamp with immobilized *E. coli* cells the polymeric film coated electrodes were rinsed with 10 mM sodium phosphate buffer (pH 7.4) and treated with 10 mg/mL lysozyme solution (in 10 mM Tris-HCl buffer, pH 8.0, with 1 mM EDTA) for 30 min to remove possible bacterial residues remaining from the imprinting process. Finally, the electrode was immersed in 1-dodecanethiol (10 mM in ethanol) for 20 min in order to fill the pinholes on the surface of the electrode.

2.4. Characterization of glass slides and gold electrodes

Gram staining was performed in order to confirm the immobilization of bacterial cells to the modified surface of glass slides (Brown, 2005). *E. coli* imprinted surfaces of gold electrodes were characterized by CV, AFM, SEM.

CV analysis was performed using a potentiostat/galvanostat (Autolab PGSTAT 12, EcoChemie, Utrecht, Netherlands) controlled by the 4.8 version GPES Software. The three electrode electrochemical flow cell used in the experiments consisted of counter electrode which completes the cell circuit, Ag/AgCl electrode which has a constant electrochemical potential and used for measuring the potential of working electrode and working electrode which has a gold surface modified in order to form an affinity surface. The electrode solution was prepared with KCl solution (0.1 M) containing 10 mM $K_3[Fe(CN)_6]$ (Mahadhy et al., 2014). Cyclic voltammetry was carried out by sweeping the potential between -0.3 and 0.8 V at a rate of 0.1 V s^{-1} to interpret the degree of insulation of the electrode surface after each step.

AFM (Veeco Instruments Inc, USA) analysis was performed in tapping mode. The electrode was attached to AFM disk using double sided carbon tape. The experimental parameters were 10 μm x10 μm of scanning area and 2.5 μm of vertical examination area.

SEM observations of the electrodes were done by Quanta 400F Field Emission SEM (USA). After cleaning of the electrode with distilled water, it was dried with nitrogen gas and then, coated with Au/Pd.

2.5. Real time *E. coli* detection with the microcontact- *E. coli* imprinted electrode

The measurements of capacitance were carried out by the automated flow-injection system (CapSenze Biosystems AB, Lund, Sweden) using current pulse method (Erlandsson et al., 2014). The *E. coli* imprinted electrode was placed in the flow cell in a continuous flow system. The capacitance was calculated as a function of time from the resulting potential profile.

Prior to analysis, as regeneration agents, pure ethyl alcohol, 10 mg/mL lysozyme solution (in 10 mM Tris-HCl buffer, pH 8.0, with 1 mM EDTA) and 50 mM glycine-HCl, pH 2.5 were injected to the system for cleaning with treatment lasting for 15 min for each agent, and these

steps were repeated between each analyte injection. Gram-negative bacterial cell walls can be disrupted by lysozyme, but it is necessary to pretreat by some procedure for the removal of the outer membrane. Ethyl alcohol treatment was applied for the removal of lipopolysaccharide layer which is the main component of the outer membrane of Gram negative bacteria. Glycine-HCl pH 2.5 was selected for regeneration of the sensor due to its ability to remove lysozyme.

Sodium phosphate buffer (10 mM, pH 7.4) was used as running buffer by the pump at a flow rate of 100 μL/min. After equilibration with this buffer, a stable baseline was monitored and then, *E. coli* suspensions of different concentrations (1.0×10^2–1.0×10^7 CFU/mL) prepared were injected consecutively (500 μL, with a flow rate of 100 μL/min). The decrease in registered capacitance corresponds to the binding of *E. coli* cells to the imprinted cavities on the electrode surface and the change in the capacitance was calculated automatically by CapSenze Smart Software (CSS). All the measurements were performed in triplicates.

Non imprinted (NIP) electrodes were prepared following the similar procedure as that of the MIP ones except for the immobilization step of *E. coli* to the surface of glass slides. The NIP electrodes were used in order to monitor effect of imprinting of the *E. coli* cells. The responses of these electrodes to the *E. coli, B. subtilis*, *S. aureus* and *S. paratyphi* strains were evaluated.

2.6. Selectivity and cross-reactivity of the *E. coli*- imprinted electrode

The template binding properties (selectivity and specificity) of capacitive system were determined by the responses to *B. subtilis*, *S. aureus* and *S. paratyphi* strains. The bacteria were included based on their properties: *B. subtilis* has different cell wall structure but similar morphology, *S. aureus* has different cell wall structure and morphology, *S. paratyphi* has similar cell wall structure and morphology. The bacterial suspensions were injected one by one and also, mixed suspensions of all 4 organisms were applied in order to determine cross reactivity. The concentrations of each organism were kept constant at 1.0×10^4 CFU/mL. NIP-electrodes were used as mentioned above in order to indicate imprinting efficiency.

2.7. Real sample experiments and reusability

Experiments were also performed with river water and apple juice samples which were diluted 10 times with running buffer in order to reduce the media effect and then spiked with *E. coli* at a range of concentrations (1.0×10^2-1.0×10^4 CFU/mL). *E. coli* was detected repeatedly, using equilibration-injection-regeneration cycles, for 70 times. The reusability of the assay was evaluated by monitoring the change in capacitance at the same concentration of standard *E. coli* suspension (1.0×10^4 CFU/mL).

3. Results and discussion

3.1. Preparation and characterization of *E. coli* imprinted electrodes

In the process of polymer preparation, MAH (histidine containing specific monomer) was selected as metal-chelating ligand in order to introduce selectivity against certain amino acid residues present on the cell wall. Cu(II) ions having affinity towards proteins were complexed with MAH (Bereli et al., 2011; Odabaşı et al., 2001). Then, *E. coli*-imprinted Poly(2-hydroxyethyl methacrylate-co-N-methacryloyl-L-histidinemethylester) poly(HEMA-MAH) polymer crosslinked with EGDMA was synthesized via UV polymerization. The interaction between *E. coli* and polymeric structure was based on the affinity interaction as well as sterical matching between the imprinted cavities on the sensor surface and the cells. Molecularly imprinted polymers (MIPs), described as artificial antibodies, have synthetic selective recognition sites similar to those of natural receptors (Bole and Manesiotis, 2015; Ye, 2015). Even though natural antibodies have high selectivity and sensitivity, they tend to denature in conditions outside the physiological range (Bole and Manesiotis, 2015). The prepared polymeric material in this study provides selectivity with chemical/physical stability, reusability and cost effectiveness due to the unique characteristics of the mixture of monomers used (Yilmaz et al., 2015). *E. coli*-imprinted capacitive electrodes were prepared using microcontact imprinting by bringing the modified surface with the vinyl groups of the electrode and the monomer solution in contact with the *E. coli*-immobilized on the glass slide like a sandwich (Figure 1). CV, AFM and SEM analysis were used for the characterization of bare and *E. coli*-imprinted capacitive electrodes.

CV is an electrochemical technique which provides information regarding the degree of insulation and enables checking the formation of insulating layers. Electropolymerization is an approach applied for both obtaining insulation and introduction of reactive groups. The electropolymerization of tyramine resulted in forming a thin layer on the electrode surface with concomitant introduction of free amine groups (Hedström and Mattiasson, 2015). The electrochemical polymerization of tyramine has gained great interest in the application studies of biosensors because it provides free amine groups that can be used for immobilization of biorecognition molecules (Ertürk et al., 2014; Gutierrez et al., 2015; Mahadhy et al., 2014). Therefore, tyramine was selected as monomer for creating insulating films in this study. As seen from Figure 2A and B, the electrochemical behaviour of the electrode was measured after each modification step. In Figure 2A, reversible oxidation and reduction of the redox couple on the bare gold electrode surface could be observed (curve a). After polytyramine layer was formed, a noticeable decrease was registered in the redox peaks which shows markedly reduced transmission of the conducting ions to the electrode surface (curve b). In the following step, the redox peaks decreased once again with the treatment of acryloyl chloride (curve c). After polymerization was performed, another decrease was registered in the redox peaks (curve d). Lastly, treatment with 1-dodecanethiol as a blocking agent resulted in a considerable reduction of the redox currents and disappearance of the redox peaks. Figure 2B shows expanded voltammograms after treatment with 1-dodecanethiol (curve d). The results obtained from cyclic voltammetry indicate successive insulation of the electrode. AFM images of the bare surface and *E. coli*-MIP electrode are shown in Figure 2C and D, respectively. As seen from these figures a rough polymeric surface was formed on the gold electrode. SEM images in Figure 3A, B, C and D also show

the change in the morphology of the polymeric surface on the *E. coli*-MIP electrode in different magnifications, Figure 3B, C and D also show the captured cells.

3.2. Real-time detection of *E. coli*

In the sensorgram graph (Figure 4A), the first peak indicates the regeneration, and after *E. coli* injection, binding of *E. coli* to the recognition cavities produces a change in capacitance (ΔC) which was calculated by the CapSenze Smart Software. As can be seen from the calibration curve (Figure 4B), the decrease in capacitance increased with the increasing concentrations of *E. coli*. Signals were registered every minute. An average of five capacitance values after a stable signal had been registered was used as the analytical signal. The plot of the change in capacitance versus the log concentrations of *E. coli* shows linear relationship in the concentration range 1.0×10^2–1.0×10^7 CFU/mL. The corresponding linear regression equation was given, $y = 1287.2x + 1928$ ($R^2 = 0.9336$) and the limit of detection (LOD) was determined to be 70 CFU/mL, based on IUPAC guidelines. Table 1 shows the a comparison of different methods for *E. coli* detection in literature. In comparison with the LOD values indicated in these studies, the LOD value obtained from our study is one of the lowest values in literature. Capacitive biosensors are sensitive and provide analytical stability. These features make these systems unique and promising candidates as alternatives to optical detection methods used.

3.4. Selectivity and cross-reactivity of the *E. coli* imprinted electrode

The selectivity of capacitive system to *B. subtilis*, *S. aureus* and *S. paratyphi* strains was evaluated. Figure 5A indicates that (ΔC) values obtained from injections of all the tested bacterial strains were lower than that of *E. coli*. Among the bacterial strains, the highest (ΔC) value was recorded after the injection of *S. paratyphi.* This result can be attributed to two major factors: (i) *S. paratyphi* is a rod-shaped Gram-negative bacteria of the Enterobacteriaceae family like *E. coli*, and (ii) has a considerable size similarity to that of *E. coli*. On the other hand, the lowest selectivity coefficient was obtained for *S. paratyphi* (Table 2).

The determination of cross-reactivity is essential in order to verify the specificity of proposed sensor. For this purpose, the cross-reactivity studies which have a significant effect on the performance of the sensor were carried out using pre-mixed suspensions of *E. coli*, *B. subtilis*, *S. aureus* and *S. paratyphi* strains. The *E. coli* imprinted capacitive electrode has a high degree of selectivity compared to other bacterial strains with cross-reactivities of 23.9-58.4% against pre-mixed suspensions of all bacterial strains. The cross-reactivity results showed that *E. coli* can be effectively detected in a pre-mixed suspension containing the other bacterial strains. However, a lower (ΔC) value was registered for *E. coli* in this case compared to that of singular manner.

All bacterial strains other than *E. coli*when exposed to the sensor chip yielded low capacitance in both MIP and NIP electrodes (Figure 5B). Higher responses against *E. coli* pointed out the unique feature of recognition cavities formed during the imprinting process.

Therefore, the recognition cavities having a memory of shape and chemical properties made it possible to obtain the highest responses against *E. coli* under both singular position and competitive conditions. The responses against competitive bacterial strains were higher in imprinted sensors compared with those of non-imprinted ones because of the similar characteristics of bacterial surfaces. Actually, there was little if any response against competitive bacterial strains and they are negligible in contrast with the responses against *E. coli* (Figure 5B).

3.5. Real sample experiments and reusability

Experiments of *E. coli* detection were carried out with river water (Figure 6A) and apple juice samples (Figure 6B) due to enabling ambient media for bacterial growth to investigate the real-time applicability of the developed sensor. Figure 6A and B shows the increase in ΔC value with the increasing concentrations of *E. coli* in river water and apple juice samples, respectively. As seen in Table 3, the prepared capacitive sensor has the ability to detect *E. coli* with a recovery of 81-97%. The efficiency of *E. coli*-imprinted electrode was kept about the similar level during 70 injections.

In literature, there are many studies focusing on successful imprinting of various small molecules (Bole and Manesiotis, 2015) and even demonstrating the applicability of imprinting microorganisms (Bacskay et al., 2006; Cohen et al., 2010; Dickert and Hayden, 2002; Dickert et al., 2004). However, there is still an ongoing challenge on the whole cell imprinting (Bole and Manesiotis, 2015). Carturan et al. (1989) developed whole cell imprinted inorganic sol-gel matrices via encapsulation of *S. cerevisiae* for monitoring environmental protection and metal recovery. After imprinting of whole cells by entrapment in sol-gel (Chia et al., 2000), surface imprinting have become superior and the first report including whole cell imprinting in sensor applications was published by Dickert and Hayden (2002). In their study, a quartz crystal microbalance (QCM) system consisting of cell-imprinted sol-gel matrix was developed for yeast detection within the detection ranges of 10^4–10^9cells/mL. *S. cerevisiae* was imprinted onto polyurethane surface providing selectivity to the target cells under flow conditions (Dickert and Hayden, 2002). The same research group also improved polymer coated QCM sensor with the surface imprinting method (Dickert et al., 2004). Tokonami et al. (2013) generated a sensor consisting of molecularly imprinted electrode created by using a QCM electrode coated with overoxidized polypyrrole (OPPy) film for the detection of *P. aeruginosa* at cell concentrations ranging from 10^3 to 10^9 CFU/mL. In another study, a QCM sensor was developed with artificial receptors relying on surface imprinted polyurethanes in order to detect *E. coli* in aqueous solution and the detection limit was found to be in the range of 0.1 mg/mL (Findeisen et al., 2012). Yilmaz et al. (2015) created both surface plasmon resonance (SPR) and QCM biosensors using microcontact imprinting for whole-cell detection of *E. coli*.

The colloidal behaviour of bacteria in water can be attributed to their physicochemical properties that influence the attachment of bacteria to surfaces. Several factors such as surface charge, hydrophobicity, pH, temperature, contact time and cell concentration

influence their attachment (Starosvetsky et al., 2012). The cell wall of Gram negative bacteria contains some chemical functional groups such as, phosphate, hydroxyl, amino and carboxylate groups. The recognition of *E. coli* took place by a concerted action with all the different monomers, also the cross-linker involved. The functional group of MAH in the imprinted film and the shape-complementary cativities to *E. coli* formed in the biofilm had at its surface groups from the monomers that had interacted with the surface of the microbial cell before polymerization. The large surface of the cell allows many interactions to take place, and even if many of them are weak individually, together they form strong interactions. One can assume that monomers with more pronounced functionalities, in this case MAH will have stronger interactions than e.g. HEMA and the crosslinker EGDMA, and therefore also be more important for the selectivity in the interactions. Differentiation between same and different bacterial morphologies has to be taken into account for selectivity experiments carried out with MIP and NIP electrodes. In a previous study aimed at *E. coli* detection, SPR and QCM sensors consisting of microcontact imprinted and non-imprinted polymers were confirmed by their responses against *Bacillus sp*. and *Staphylococcus sp*. The results obtained from the responses of these two bacterial strains were similar to those of our results. Furthermore, in this study, the responses of prepared system was tested against *Salmonella sp.* which has shape and size similarity with *E. coli*. The size distribution should be taken into consideration, while the bacterial cells were docking to the cavities during rebinding (Tokonami et al., 2013). From the point of differentiation between similar bacterial morphology, the cavities could be easily occupied by *Salmonella sp.* because of having a similar width and length to that of *E. coli.* Both *E. coli* and *Bacilus sp*. have rod-like morphology, *Bacilus sp.* is larger than *E. coli.*

In a previous study, the real time experiment was performed with apple juice to confirm whether the prepared system works and the measurable concentration range in apple juice was pointed out between 10^7 to 10^9 CFU/mL for tested bacterial strain (Tokonami et al., 2013). Yılmaz et al. (2015) also preferred the apple juice spiked with *E. coli* at different concentrations in the range of 0.5–4.0 McFarland (Donay et al., 2007) (approx. 1.5×10^8 to 12×10^8 CFU/mL) as real sample in order to verify the proposed SPR and QCM sensors for detecting *E. coli*.

In this study, both *E. coli* spiked (1.0×10^2 to 1.0×10^4 CFU/mL) apple juice and river water samples were used in real sample experiments for determining the applicability of the capacitive sensor. In comparison to other studies performed before, the result obtained from our experiments demonstrated that developed sensor was capable of quantifying bacterial cells even when they were at low concentrations.

It is important to investigate recovery in order to determine the sensitivity of the prepared systems. Cohen et al. (2010) indicated 10% recovery of *Deinococcus radiodurans* and *E. coli*. According to a previous study, the generated sensor could differentiate *E. coli* from other bacterial cells in the complex matrix of waste water with good recovery (92-98%) (Roy et al., 2015). In our study, we also investigated capturing *E. coli* from river water sample and achieved a recovery 81-97%.

4. Conclusion

The objectives of this investigation were threefold. Our first objective was to evaluate the possibility to design a sensor surface selective for a certain microorganism using microcontact imprinting. The second objective was to utilize such an imprinted sensor chip in a capacitive biosensor, and the third objective was to study the selectivity of the biosensor for different microorganisms. All three objectives were met since the microcontact imprinted sensor gave sensitive and selective responses when challenged with the print organism alone and in competition with other microorganisms.

Use of the described sensor has potential to serve as promising candidate for monitoring *E. coli* in contaminated water or food supplies In the future, it can also be developed and employed for the rapid detection of pathogen microorganisms in real samples and make it possible to devise appropriate control strategies..

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Figure 1. Schematic representation of microcontact imprinting of *E. coli* onto the polymer modified surfaces. (A) preparation of electrode surface, (B) preparation of bacteria stamps, (C) production of the microcontact imprinting.

Figure 2. Characterization of *E. coli* imprinted electrode surfaces with CV and AFM. (A) Cyclic voltammograms recorded in a solution of 100 mM KCl containing 10 mM $K_3[Fe(CN)_6]$ using: bare electrode (black-a), gold electrode after electropolymerization of tyramine (green-b), gold electrode after surface modification with acryloyl chloride (blue-c), after treatment with 1-dodecanethiol (purple-d). The scan range was from −0.3 to 0.8 V (vs. Ag/AgCl), at a scan rate of 0.1 V s^{-1}, (B) Cyclic voltammograms recorded in a solution of 100 mM KCl containing 100 mM $K_3[Fe(CN)_6]$ using: gold electrode after electropolymerization of tyramine (black-a), gold electrode after surface modification with acryloyl chloride (green-b), gold electrode after polymerization (blue-c), after treatment with 1-dodecanethiol (purple-d). The scan range was from −0.3 to 0.8 V (vs. Ag/AgCl), at a scan rate of 0.1 V s^{-1}, (C) AFM image of bare electrode, (D) AFM image of *E. coli* imprinted electrode.

Figure 3. SEM images of bare gold electrode (A)-50000X, and *E. coli* imprinted electrode (B)-10000X, (C and D)-50000X magnifications.

Figure 4. (A) Capacitive sensorgram of the *E. coli* imprinted electrode (concentration of *E. coli*: 1.0×10^7 CFU/mL), ΔC: change in capacitance, (B) Calibration curve of *E. coli* obtained at a concentration range of (1.0×10^2–1.0×10^7 CFU/mL) under optimum conditions, LOD: Limit of detection (Flow rate: 100 µL/min; sample volume: 500 µL; running buffer: 10 mM sodium phosphate, pH: 7.4; regeneration buffer: pure ethyl alcohol, 10 mg/mL lysozyme solution (in 10 mM Tris-HCl buffer, pH 8.0, with 1 mM EDTA) and 50 mM glycine-HCl, pH 2.5; T 25°C.

Figure 5. Figure (A) Selectivity of microcontact-*E. coli* imprinted capacitive biosensor against competing bacterial strains; *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella paratyphi* in singular and competitive manner (B) Imprinting efficiency of the microcontact-*E. coli* imprinted electrode vs. NIP (non-imprinted) electrode (concentration of bacterial suspensions: 1.0×10^4 CFU/mL; flow rate: 100 µL min-1; sample volume 500 µL; running buffer 10 mM sodium phosphate, pH 7.4; regeneration buffer: pure ethyl alcohol, 10 mg/mL lysozyme solution (in 10 mM Tris-HCl buffer, pH 8.0, with 1 mM EDTA) and 50 mM glycine-HCl, pH 2.5; T 25 ºC).

Figure 6. Real sample experiments (A) Real-time responses of *E. coli* imprinted capacitive biosensor indicating the sequential injection of *E. coli* spiked river water sample, Sample 1: 1.0×10^2 CFU/mL, Sample 2: 1.0×10^3 CFU/mL, Sample 3: 1.0×10^4 CFU/mL, (B) Real-time responses of *E. coli* imprinted capacitive biosensor with *E. coli* spiked apple juice sample (flow rate: 100 µL/min; sample volume: 500 µL; running buffer: 10 mM sodium phosphate, pH: 7.4; regeneration buffer: pure ethyl alcohol, 10 mg/mL lysozyme solution (in

10 mM Tris-HCl buffer, pH 8.0, with 1 mM EDTA) and 50 mM glycine-HCl, pH 2.5; T 25°C

Table 1. Comparison of different methods for *E. coli* detection.

Bacterial strain	Methodology	Limit of detection (LOD)	Reference
E. coli	Electromagnetic trap magneto-resistive sensor	1.29×10^8 CFU/mL	(Li and Kosel, 2014)
E. coli	SPR plastic optical fiber	10^4 -10^8 CFU/mL	(Wandermur et al., 2014)
E. coli	Potentiometric aptamer based sensor	26×10^8 CFU/mL	(Zelada-Guillén et al., 2010)
E. coli	Label-free impedimetric biosensor based on lectin functionalized mixed self assambeled monolayer	75 cell/mL	(Yang et al., 2015)
E. coli	Metal enhanced electrochemical detection and label-free SPR	3 CFU/mL	(Yazgan et al., 2014)
E. coli	SPR using lectin	3×10^3 CFU/mL	(Wang et al., 2013)
E. coli	SPR (substractive inhibition assay)	3×10^5 CFU/mL	(Wang et al., 2011)
E. coli	SPR (immunosensor)	10^3 CFU/mL	(Subramanian et al., 2006)
E. coli	MIP based Quartz crystal microbalance (QCM)	0.1 mg/mL	(Findeisen et al., 2012)
E. coli	Microcontact imprinting based QCM	1.54×10^6 CFU/mL	(Yilmaz et al., 2015)
E. coli	Microcontact imprinting based Surface plasmon resonance (SPR)	3.72×10^5 CFU/mL	(Yilmaz et al., 2015)
E. coli	Microcontact imprinting based capacitive biosensor	70 CFU/mL	This study

Table 2. Selectivity coefficients of *E. coli*-MIP and NIP electrodes [Δ: Capacitance change for the *E. coli*-MIP and NIP electrodes, k: selectivity coefficient for *E. coli* versus competing bacterial strains, k′: relative selectivity coefficient for *E. coli*-MIP electrode versus NIP electrode].

Bacterial strains	Capacitance change, ΔC Imprinted	Capacitance change, ΔC Non-imprinted	Selectivity coefficient, *k* Imprinted	Selectivity coefficient, *k* Non-imprinted	Relative selectivity coefficient, *k'*
E. coli	6649	554	-	-	-
B. subtilis	2120	563	3.14	0.98	3.20
S. aureus	2003	466	3.32	1.19	2.79
S. paratyphi	2230	610	2.98	0.91	3.27

Table 3. Recovery tests for *E. coli* spiked in river water sample.

River water sample	Added (CFU/mL)	Found)±S.D (CFU/mL.	Recovery (%)	RSD (%)
Sample 1	1.00×10^2	$(0.97 \pm 0.03) \times 10^2$	97	1.3
Sample 2	1.00×10^3	$(0.81 \pm 0.04) \times 10^3$	81	1.3

Sample 3	1.00 x 10^4	(0.97 ±0.03) x 10^4	97	1.2

[#] S.D. = Standard deviation

Highlights:

- a capacitive biosensor for quantifying microbial cells was developed
- microcontact imprinting was used to make the sensor surface
- selectivity for the imprinted cell was demonstrated
- sensitivity: LOD 70 CFU per mL.

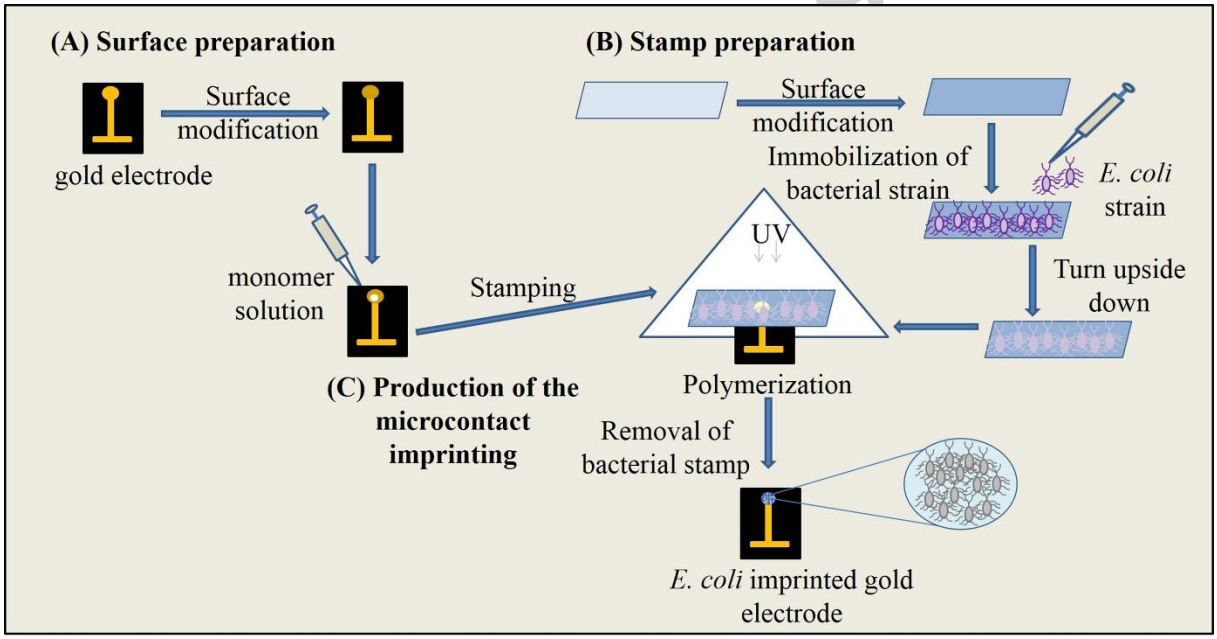

Figure 1.

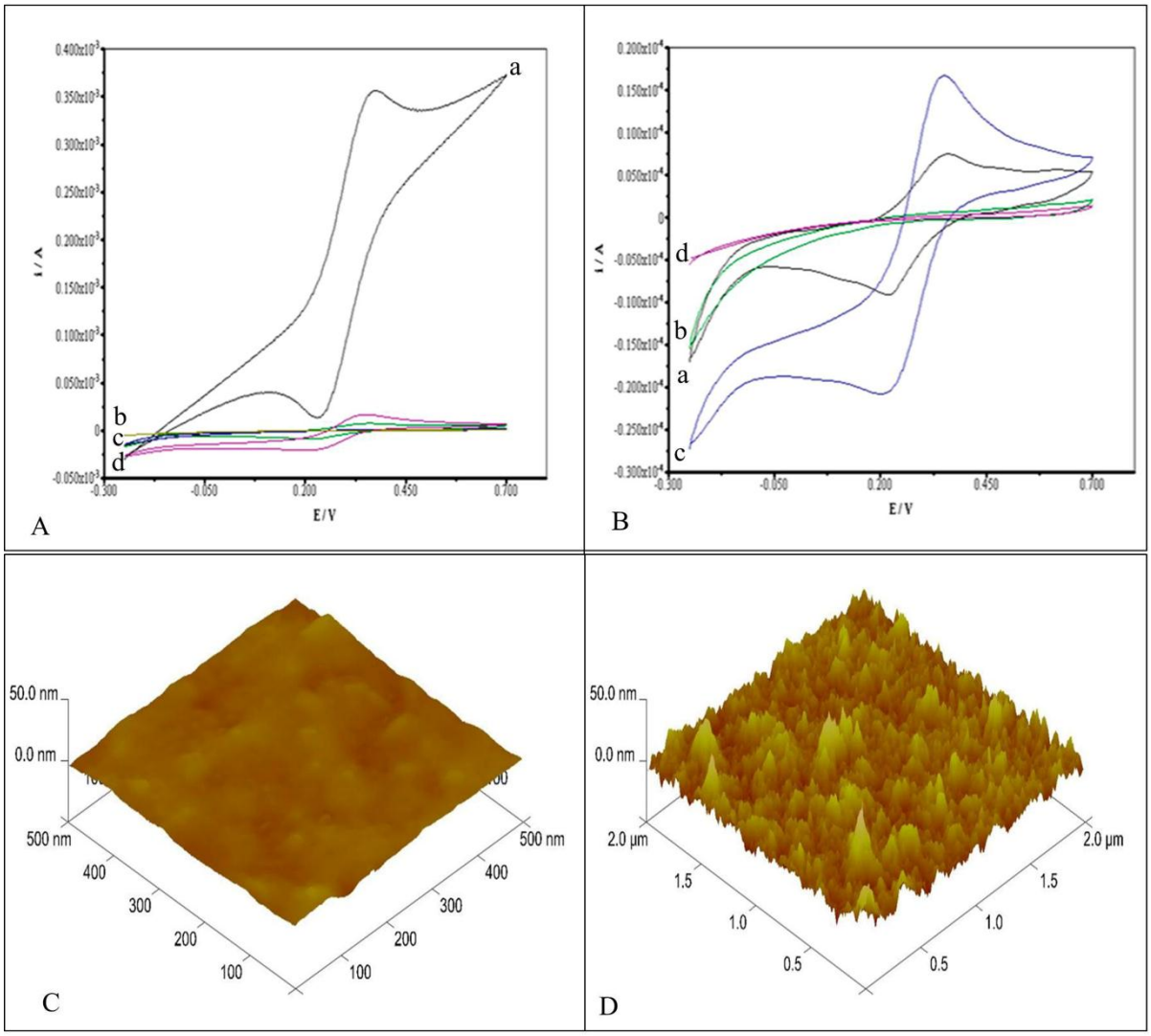

Figure 2.

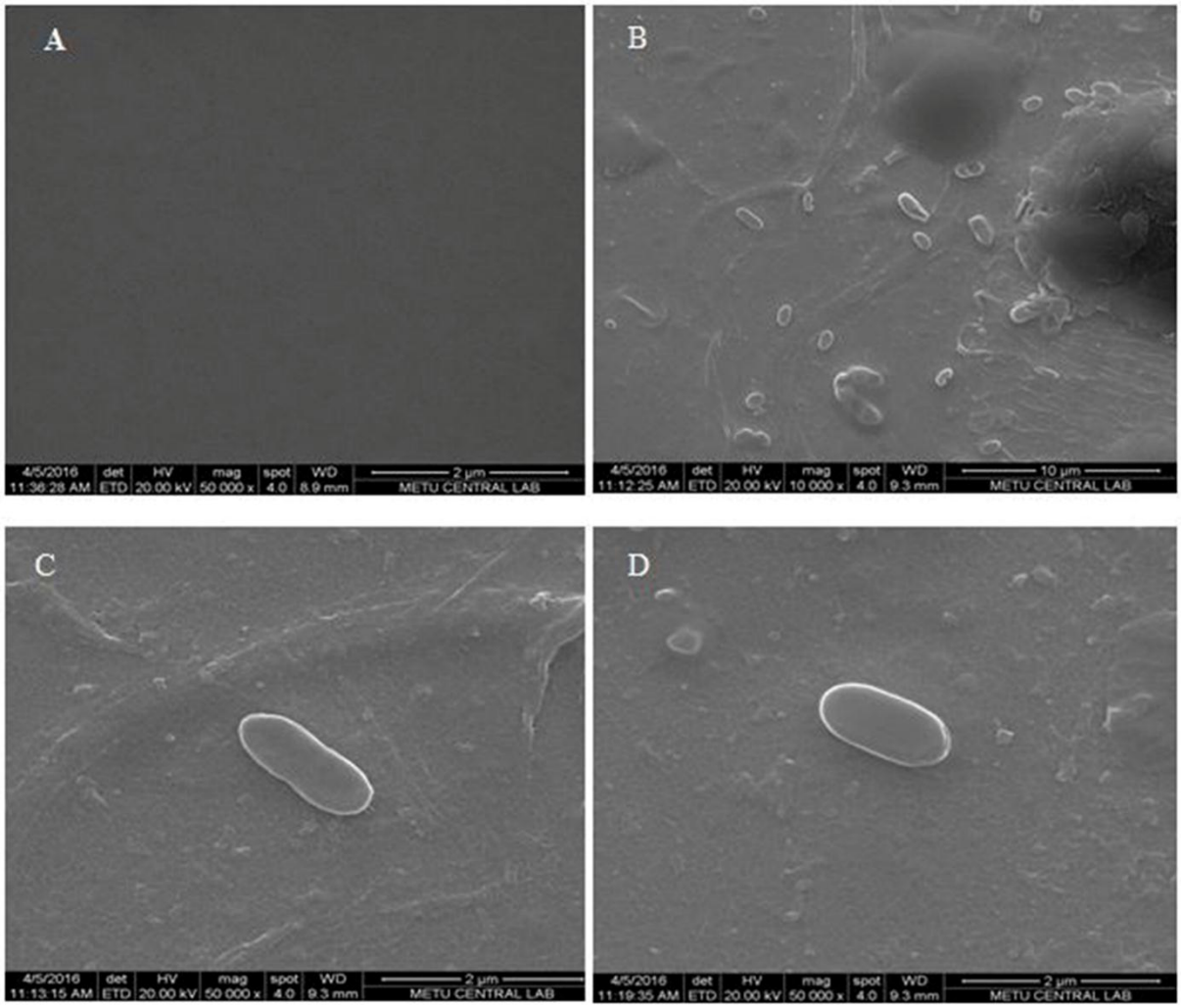

Figure 3.

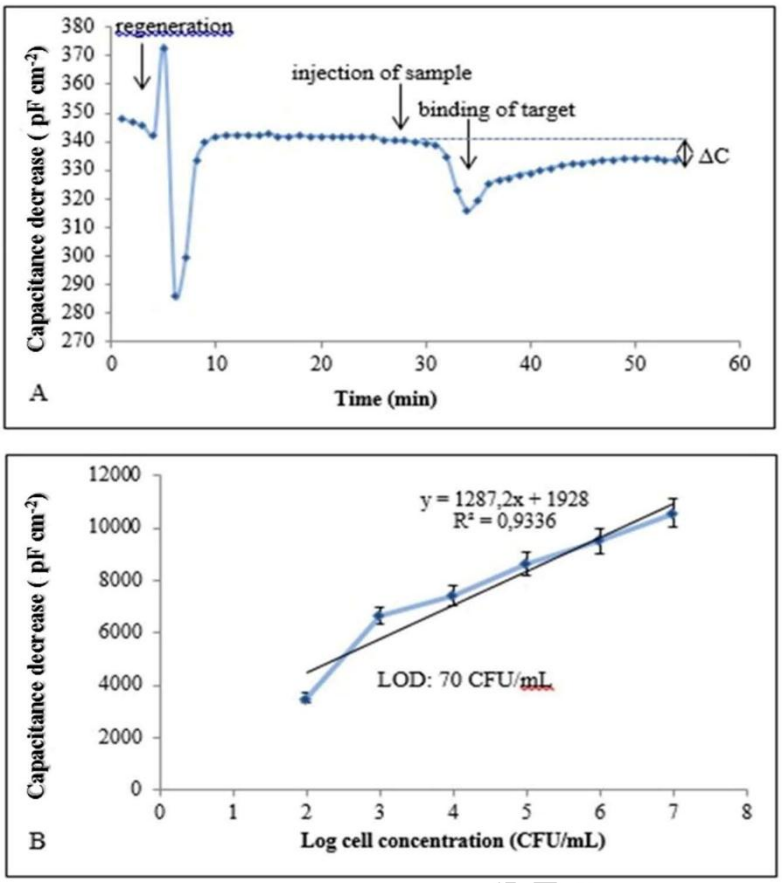

Figure 4.

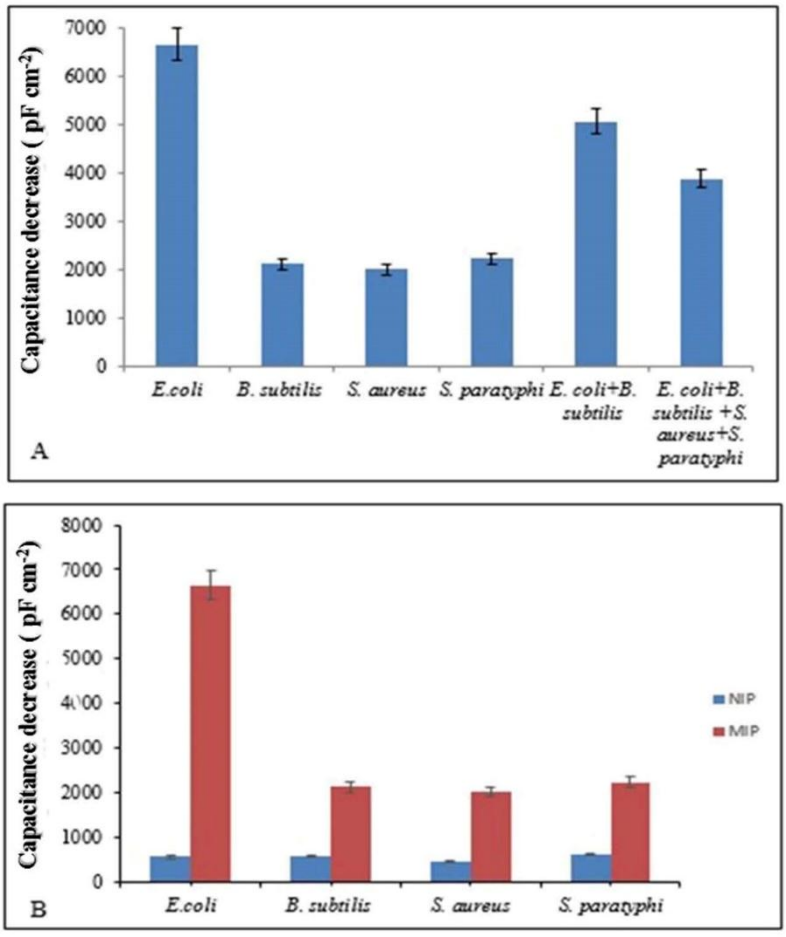

Figure 5.

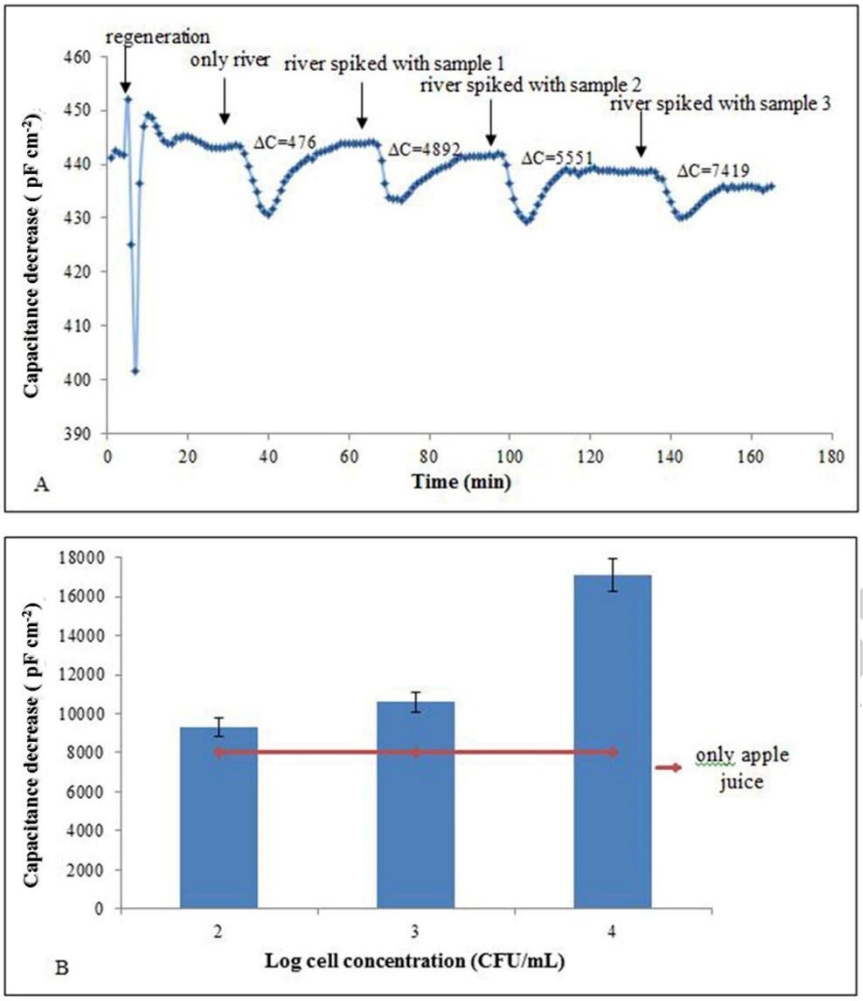

Figure 6.