Journal of Food Protection, Vol. 72, No. 3, 2009, Pages 591–600

Concentration and Detection of *Salmonella* in Mung Bean Sprout Spent Irrigation Water by Use of Tangential Flow Filtration Coupled with an Amperometric Flowthrough Enzyme-Linked Immunosorbent Assay

R. McEGAN,[1] T. J. FU,[2] AND K. WARRINER[1]*

[1]*Department of Food Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada; and* [2]*U.S. Food and Drug Administration, National Center for Food Safety and Technology, Summit-Argo, Illinois 60501, USA*

MS 08-168: Received 5 April 2008/Accepted 3 July 2008

ABSTRACT

The development of a culture-free method for *Salmonella* screening of spent irrigation water derived from sprouting mung bean beds is described. The system used tangential flow filtration (TFF) to nonspecifically concentrate cells from large (2- to 10-liter) sample volumes. The retentate (100 ml) from the TFF was then flowed over an anti-*Salmonella* antibody–modified cellulose acetate membrane. The captured *Salmonella* was detected by reacting with a secondary anti-*Salmonella* and goat anti-rabbit biotin labeled antibody, followed by avidin-tagged glucose oxidase. The hydrogen peroxide generated from the enzymic oxidation of glucose was amperometrically detected at an underlying platinum electrode. It was found that 10 liters of *Salmonella* suspensions of 2 log CFU/ml could be concentrated to 4 log CFU/ml with 60% recovery regardless of the flow rate (112 to 511 ml/min) or transmembrane pressure (0 to 20 lb/in^2) applied. The solids content of spent irrigation water negatively affected the filtration rate of TFF. This was most evident in spent irrigation water collected in the initial 24 h of the sprouting period, where the solids content was high (4,170 mg/liter) compared with samples collected at 96 h (560 mg/liter). Trials were performed using mung bean beds inoculated with different *Salmonella* levels (1.3 to 3.3 log CFU/g). By using the optimized TFF and flowthrough immunoassay it was possible to detect *Salmonella* in spent irrigation water at levels of 2.43 log CFU/ml within 4 h. The integrated concentration and detection system will provide a useful tool for sprout producers to perform in-house pathogen screening of spent irrigation water.

Sprouted seeds have been implicated in numerous outbreaks of foodborne illness *(1)*. *Salmonella* remains the most common human pathogen associated with sprouts, although outbreaks linked to *Escherichia coli* O157:H7 have also been reported *(16, 27, 34, 43)*. To address the increasing number of foodborne illness outbreaks, the U.S. Food and Drug Administration issued guidelines in 1999 to enhance food safety standards within the sprout industry *(1)*. Among other measures, the guidelines recommend testing the spent irrigation water 48 h into the sprouting process for the presence of pathogens with specific reference to *Salmonella (1)*. Screening spent irrigation water is preferred over testing sprouts directly due to uniformity, ease of collection, and simplicity of analysis *(1)*. More significantly, because irrigation water runs over the sprouts, it is considered to provide a better assessment on the microbiological status of the sprouting seed bed compared to when individual sprout samples are screened *(1)*.

The protocols for sampling spent irrigation water were validated through studies using inoculated and noninoculated alfalfa (*12, 33*). The results from the studies indicated that the growth of *Salmonella* on sprouting alfalfa occurred early in the sprouting period and was homogenously distributed throughout the sprout bed *(12, 33)*. However, a study performed by Hora et al. *(15)* illustrated that contamination within sprouting mung bean beds was heterogeneously distributed and the spread of pathogens (*Salmonella* and *E. coli* O157:H7) was delayed when introduced at low levels (1 g of inoculated beans per 500 g) *(15)*. Consequently, screening spent irrigation water from a single point early in the sprouting period would not provide a reliable measure of the microbiological safety of mung bean batches *(15)*. A more effective screening method would be to screen large volumes of spent irrigation water late in the sprouting period. However, large sample volumes are incompatible with standard microbiological techniques, which typically involve preenrichment followed by selective plating. In addition, because of the time required to perform pathogen screening, spent irrigation samples need to be collected within the initial 48 h into sprouting to ensure that the results are known prior to product release *(1)*. To address such time constraints, there has been a sustained interest in rapid detection methods for screening spent irrigation water. To date the majority of studies have focused on pathogen detection methods based on immuno (latex test) or molecular methods (real-time PCR) *(22, 27, 32)*. Although such techniques are both rapid and sensitive, the sample size applied to the sensor is restricted to microliter volumes, thereby requiring a preenrichment step to increase pathogen levels to the limit of detection. In addition, culture-based techniques are unsuitable for on-site testing due

* Author for correspondence. Tel: 519 824 4120, Ext 56072; Fax: 519 824 6631; E-mail: kwarrine@uoguelph.ca.

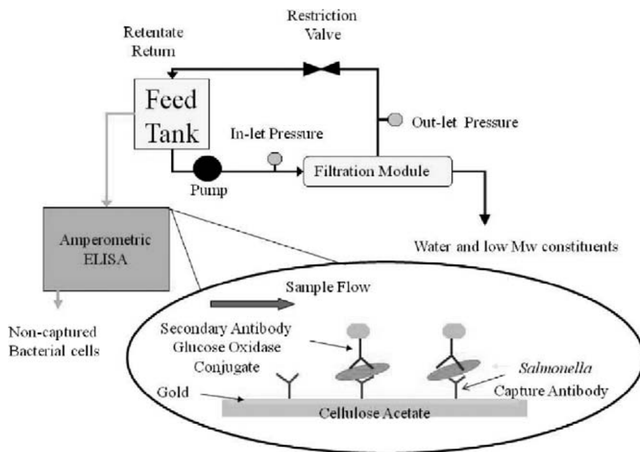

FIGURE 1. *Schematic diagram of the integrated TFF and amperometric flowthrough ELISA. The spent irrigation water sample is recirculated through a TFF unit to concentrate bacterial cells. The retentate is then flowed over capture antibodies immobilized on the surface of a cellulose acetate membrane. The captured* Salmonella *cells are detected via the addition of secondary antibody glucose oxidase conjugate.*

to the need for laboratory facilities and incurred time delay *(22)*.

A non–culture-based method has been described for screening spent irrigation water derived from alfalfa beds. The method described was based on centrifugation of spent irrigation water (400 ml) to concentrate cells followed by selective detection using PCR *(21)*. The researchers reported 99% recovery of *Salmonella* or *E. coli* O157:H7 with a lower detection limit of 2 log CFU/ml. Although sensitive, it can be considered that centrifugation and molecular techniques are too expensive for sprout producers to adopt.

In the present study an integrated system has been developed based on tangential flow filtration (TFF) in combination with a flowthrough electrochemical enzyme-linked immunosorbent assay (ELISA) to enable a sensitive, culture-free approach to screening spent irrigation water derived from mung bean sprout production (Fig. 1). TFF is a cross-flow system that enables high filtration rates of large volumes of sample without excessive pore blocking as experienced with dead-end filtration techniques *(26)*. TFF has been used extensively in the biotechnology industry to recover proteins or metabolic products from fermentations *(39)* with less attention being placed on recovering microbial biomass *(14, 26)*. However, Fu *(11)* developed a TFF sampling system for testing spent irrigation water derived from alfalfa sprout production. Here, the spent irrigation water sample collected 48 h into the sprouting period was circulated within a TFF system achieving 100-fold concentration of inoculated *Salmonella* or *E. coli* O157:H7 within 2 h *(11)*.

In the present study, the selective detection of *Salmonella* was achieved using a flowthrough electrochemical ELISA sensor. Flowthrough immune sensors have the distinct advantage over conventional ELISA in terms of greater sample volumes that can be analyzed, thereby enhancing sensitivity *(4, 36, 44, 45)*. Here, the sample is allowed to flow over a layer of antibodies to capture the target analyte and subsequently allowed to react with an enzyme conjugate. The enzymatic product of the conjugate can then be detected either colorimetrically or electrochemically. Electrochemical transduction has the advantage over optically based methods with respect to low-cost hardware, robustness, and relatively simple design, in addition to multianalyte sensing using microarrays *(25)*. The two basic formats available for electrochemical-based sensors are chromatographic (immune columns) or antibody-modified electrodes. In the chromatographic format, the cells are captured within a column and subjected to reaction with an enzyme conjugate along with substrate. The electroactive product is then eluted and detected at a downstream electrode *(25)*. The alternative approach is to directly or indirectly immobilize antibodies onto the electrode surface, thereby enabling low amounts of enzyme conjugate to be used in addition to enhanced signals due to the lower dilution of enzyme product *(25)*. However, a potential limitation of this approach is passivation of the electrode by deposition of proteins and other organic constituents from the sample matrix *(6, 28, 35)*. Therefore, a protective membrane is typically overlaid onto the electrode to prevent fouling of the electrode while at the same time allowing diffusion of the enzyme product *(20, 30)*. This is the main reason for selecting a low-molecular-weight enzymatic product, such as hydrogen peroxide, that readily diffuses across membrane films to the underlying electrode *(41)*.

The objective of this study was to develop a robust system for spent irrigation water screening that could be readily adopted by sprout producers. To mimic the worst-case scenario, the integrated system was verified using spent irrigation water derived from sprouting mung bean beds inoculated with low levels of *Salmonella* (1.3 log CFU/g) that may be encountered naturally in contaminated seeds *(23)*.

MATERIALS AND METHODS

Preparation of *Salmonella* suspensions. The *Salmonella enterica* serovars selected for study either have been implicated in sprout-related foodborne illness outbreaks or are of clinical significance. The *Salmonella* serovars used (obtained from the culture collection of the Canadian Research Institute for Food Safety, Guelph, Canada) in the study were Montevideo P2 (kanamycin resistant *(42)*), Heidelberg, Senftenberg, Typhimurium DT104, Newport, Meleagridis, and Oranienburg. The *Salmonella* organisms were cultivated aerobically in Luria-Bertani (LB) broth (Oxoid, Basingstoke, UK) at 37°C for 24 h. The cells were harvested by centrifugation (5,000 $\times$ *g* for 10 min at 4°C) and washed once in sterile saline. The pellet was finally resuspended in saline to give a final cell density of 7.0 log CFU/ml (optical density at 600 nm, 0.2).

Preparation of spent mung bean irrigation water. Batches (500 g) of mung beans (donated by a local sprout producer) were placed into a 10-liter container with a perforated base to allow drainage of irrigation water. The container was placed within a secondary vessel to collect the spent irrigation water. The mung beans were soaked overnight in 2 liters of water at 25°C. The water was removed, and sprouting continued for a further 96 h with daily irrigation using 2-liter volumes of sterile distilled water.

On each occasion the water was collected and transferred to a sterile bottle and maintained at 4°C until required.

Solids content of spent irrigation water was determined using a conductivity meter (Thermo-Fisher, Whitby, Ontario, Canada).

Enumeration of *Salmonella* in spent irrigation water derived from sprouting mung bean beds. Suspensions (500 ml) of *Salmonella* Montevideo P2 were prepared as described above. Mung beans (100 g) were steep inoculated in the bacterial suspension (7 log CFU/ml) for 20 min and subsequently transferred to blotting paper and allowed to dry overnight at room temperature (ca. 23°C). Initial loading of the mung beans was determined by placing 1 g of inoculated beans into 9 ml of 0.1% peptone and vortexed for 60 s to release the attached cells. Serial dilutions were prepared in 0.1% peptone and plated onto the LB_{KAN} agar (LB containing 30 μg of kanamycin per ml). Different weights (1, 10, or 100 g) of inoculated beans were introduced into noninoculated mung bean batches to give a total weight of 500 g. The mung beans were soaked in 2 liters of water for 24 h at 25°C to stimulate germination. The beans were transferred to 10-liter containers as described above, and sprouting continued for up to 96 h with daily irrigation using 2-liter volumes of water. *Salmonella* levels within the spent irrigation water samples were determined by preparing a dilution series and plating onto LB_{KAN} that was subsequently incubated at 37°C for 24 h. When no counts were recovered on plates, 100-ml volumes of spent irrigation water were passed through a sterile filter (47 mm, 0.4-μm pore size; Thermo-Fisher) and overlaid onto an LB_{KAN} agar prior to incubating at 37°C for 24 h.

Presence/absence tests for *Salmonella* were performed by mixing a 200-ml sample with 25 ml of 0.1% peptone water and incubating at 37°C for 24 h. Aliquots (0.1 ml) were spotted onto semisolid RV agar (Oxoid) and incubated at 42°C for 20 h. Presumptive-positive samples were plated onto XLD agar and incubated for 24 h at 37°C. The Oxoid *Salmonella* Latex Test FT0203 was used for serological confirmation of typical colonies (i.e., red colonies surrounded by brilliant red zones).

TFF. The TFF system, developed by Fu et al. *(11),* was adopted. The system uses a MiniKros module composed of hollow fiber polysulfone membranes with a 0.72-m^2 working area and 0.2-μm pore size (Spectrum Labs, Rancho Dominguez, Calif.). Other components of the TFF system were a 10-liter capacity holding chamber, Polycap 36HD pre-filter (10-μm pore size; Spectrum Labs), peristaltic pump (Masterflex Economy Pump, Cole-Parmer, Vernon Hills, Ill.), pressure gauges at the inlet and outlet of the TFF filter (Fig. 1). The sample to be concentrated was placed in the 10-liter holding chamber and recirculated in a closed loop system through the TFF unit. The flow rate was adjusted by the speed of the pump with the inlet and outlet pressures being controlled by tightening screw clamps (Thermo-Fisher) on the retentate side of the TFF.

The transmembrane pressure and flux rate were calculated using the following equations:

$$\text{TMP} = \frac{\text{feed pressure} + \text{retentate pressure}}{2} - \text{filtrate pressure}$$

$$\text{Flux rate} = \frac{\text{filtrate rate (h)}}{\text{area of the membrane (m}^2\text{)}}$$

The filtrate was collected in a secondary container, and filtration continued until the dead volume of the system (100 ml) had been attained. The percent capture was calculated using the following equation:

$$\%\ \text{capture} = \frac{\textit{Salmonella}\ \text{CFU in retentate}}{\textit{Salmonella}\ \text{CFU original suspension}} \times 100$$

Clean-in-place of TFF filter cartridge. Three sanitizers were evaluated for clean-in-place regimes for regenerating the TFF system. The sanitizers tested were sodium hypochlorite (200 mg/liter), acidified sodium chlorite (200 mg/liter); and sodium hydroxide (200 mg/liter). For each test the filter was used to concentrate a 2-liter *Salmonella* cell suspension (5 log CFU/ml). Upon completion of the concentration run the TFF was back-flushed using 5 liters of sterile distilled water followed by a 20-min circulation of 1-liter volumes of the selected sanitizer. Residual sanitizer was then removed by back-flushing with an additional 5 liters of sterile distilled water. To verify the efficacy of sanitation, a 2-liter volume of saline was circulated through the TFF until the 100-ml dead volume had been attained. The feed, retentate, permeate, final back-flush, and second retentate were screened for the presence of *Salmonella* by plating onto LB_{KAN} agar as previously described.

Construction of flow through immunoassay. The electrochemical cell consisted of a 3-mm-diameter platinum electrode with an Ag/AgCl outer electrode that served as the counter and reference (Rank Brothers, Cambridge, UK). The electrode was linked to a Solartron 1260 potentiostat (London Scientific, London, Ontario, Canada) and polarized at 0.65 V versus Ag/AgCl. The current response was recorded, and the data were processed using CorrWare (Scribner, Southern Pines, N.C.). The retentate was placed in a holding chamber as fed into the electrochemical cell via a peristaltic pump (Thermo-Fisher) operating at 0.89 ml/min. The volume above the membrane was set at 0.5 cm via a plunger which was placed into the electrochemical cell.

The working electrode was overlaid with a membrane that served as a surface to immobilize the capture antibody and semi-permeable interface to permit diffusion of hydrogen peroxide (product of glucose oxidase enzyme conjugate), as well as a means of minimizing electrode fouling. The membranes evaluated for this purpose were plasticized polyvinyl chloride (PVC), cellulose acetate, and polyphenyl ether sulfone (PES). PVC was prepared by dissolving 60 mg of PVC (molecular weight [MW], 43,000, Sigma-Aldrich, Oakville, Ontario, Canada) in 10 ml of tetrahydrofuran containing 100 μl of Tween 20. The solution was cast into a glass petri dish (100-mm diameter), and the solvent was evaporated overnight at room temperature (ca. 23°C) within a fume hood.

PES (60 mg; Sigma-Aldrich) was dissolved in 10 ml of chloroform and cast into a glass petri dish (100-mm diameter). Cellulose acetate (0.1 g) (MW, 30,000, Sigma-Aldrich) was dissolved in 10 ml of acetone and cast into a glass petri dish. In each case the solvent was allowed to evaporate at room temperature within a fume hood. The permeability of the membranes to hydrogen peroxide was assessed by overlaying the working electrode with 1.5-cm^2 sections of the test films. An aliquot (2 ml) of KCl (0.5 M) was added to the electrochemical cell that was stirred via a magnetic stirrer. The working electrode was polarized by applying 0.65 V versus Ag/AgCl, and the current was allowed to attain a steady baseline. A step calibration curve was constructed by the sequential addition of aliquots (0.1 ml) of hydrogen peroxide (1 mM; Thermo-Fisher). The response was determined by subtracting the steady-state current from the baseline.

To assess the antifouling properties of the different membranes, step calibration curves were performed in the presence of bovine serum albumin (BSA, 10 mg/ml; Sigma-Aldrich) or a 7.0-log CFU/ml heat-killed *Salmonella* suspension.

Capture antibody immobilization. A mouse monoclonal anti-*Salmonella* immunoglobulin G antibody, with specificity to the lipopolysaccharide core of groups A through E (ab20949, Abcam, Cambridge, Mass.), was immobilized onto cellulose acetate membranes by using a thiolation reaction. The reactive gold layer was deposited onto cellulose acetate membranes by sputter coating at 15 mV for 2 min. The monoclonal anti-*Salmonella* antibody was diluted to 0.5 mg/ml in phosphate-buffered saline (PBS). Three cross-linker solutions (all obtained from Sigma-Aldrich) were used to covalently attach a thiol group to the antibody, specifically, 20 mM dimethyl-3,3′-dithiobispropionimidate·2HCl (DTBP) (20 mM bicarbonate buffer, pH 9.0); 20 mM 3,3′-dithiobis(sulfosuccinimidylpropionate; DTSSP) (5 mM sodium citrate buffer, pH 5.0); and 20 mM each of *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) and sulfosuccinimidyl 6-[3-(2-pyridyldithio)propionamido] hexanoate (sulfo-LC-SPDP) in sterile water. Aliquots (3 μl) of each of the antibody and the three cross-linker solutions were gently mixed and incubated at room temperature for 1 h. To reduce the disulfide bond of the now-thiololated antibody, 2 μl of dithiothreitol (0.1 M sodium acetate buffer, 0.1 M NaCl, pH 4.5) was added to the mixture and allowed to react for 30 min. The solution was then spread onto the gold-layered cellulose acetate membrane and allowed to dry at room temperature for 1 h. The antibody-coated cellulose acetate membrane was then washed consecutively with PBS and sterile distilled water.

Verification of antibody immobilization was performed by flowing a 100-ml *Salmonella* suspension (7 log CFU/ml) over the antibody-modified cellulose acetate with flow rates of 0.58, 0.69, or 0.89 ml/min. The membrane was then rinsed with sterile water to remove loosely attached cells. The captured cells were fixed onto the membrane by using 2% glutaraldehyde and 1% osmium oxide followed by dehydration in ethanol solutions as described by Gamliel *(13)*. The membrane was then sputter coated with gold and viewed under a Hitachi S-3700N scanning electron microscope (Hitachi, Pleasanton, Calif.).

Capture efficiency of the flowthrough immune sensor was determined using 100-ml *Salmonella* suspensions diluted from 7 to 2 log CFU/ml. The suspension was circulated around the flow system at a rate of 0.89 ml/min (total time, 2 h). Samples (1 ml) were removed at the start and end of the flow cell process and plated onto LB_{KAN} or XLD prior to incubation at 37°C for 24 h. The capture efficiency was calculated by comparing *Salmonella* in the original suspension with levels retained within the suspension following the completion of the circulation through the immune sensor. The degree of nonspecific binding was determined by using gold-sputtered cellulose acetate membranes containing no anti-*Salmonella* antibodies.

Electrochemical detection of *Salmonella*. *Salmonella* cells captured on the antibody modified cellulose acetate membranes were allowed to react for 20 min at room temperature with 25 μl of a secondary antibody (rabbit polyclonal with affinity for O and H antigens of *Salmonella,* 1 mg/ml; ab35156). The reaction cell was washed with five 1-ml volumes of saline to remove unbound antibody prior to addition of 100 μl of biotin-labeled goat polyclonal anti-rabbit (0.02 mg/ml; ab6720, Abcam). The antibody was allowed to attach for 20 min at room temperature prior to rinsing the cell with saline. Finally, 20 to 120 μg of glucose oxidase avidin-conjugated glucose oxidase (Vector Laboratories, Burlington, Ontario, Canada) was applied to the membrane and allowed to bind to the biotin moiety of the anti-rabbit polyclonal for 40 min. The reaction cell was rinsed with sterile water to remove unattached glucose oxidase, and 1 ml of electrolyte (0.1 M KCl) was added. The background current was allowed to attain a steady-state value prior to the addition of 1 ml of glucose (1 M). The increase in current was recorded and subtracted from the background to give the sensor response.

The concentration of active units of glucose oxidase antibody conjugate was determined using a dianisidine-peroxidase assay. The assay consisted of 1.25 ml of dianisidine buffer mixture (0.1% [wt/vol]; Sigma-Aldrich), 100 μl of peroxidase (20 Purpurgalin U/ml) (Sigma-Aldrich), and 1 μl of the conjugated glucose oxidase. The reaction was started by the addition of 150 μl of 18% (wt/vol) glucose, and the increase in $A_{460\text{ nm}}$ was recorded over 5 min using a Shimadzu spectrophotometer (Thermo-Fisher). The amount (in units per milligram of enzyme) was calculated as follows:

$$\text{Units/mg} = \frac{\Delta A_{460\text{ nm}}/\text{min}}{11.3 \times \text{enzyme (mg)/reaction mixture (ml)}}$$

Statistical analysis and experimental design. Except where otherwise stated, all tests were repeated three times. Means generated were analyzed by analysis of variance and Tukey's honestly significant difference test. In all cases, the level of significance was set at P values of <0.05.

RESULTS AND DISCUSSION

Concentration of *Salmonella* suspensions by using TFF. For optimization studies, 10 liters of 0.8% (wt/vol) saline inoculated with 2 log CFU of *Salmonella* per ml (6 log CFU/10 liters) was circulated through the TFF operating at different flow rates and transmembrane pressure (TMP). The suspension was concentrated within the TFF system until the dead volume (100 ml) had been attained, which took 21 min to 3 h depending on the applied flow rate and pressure.

The average concentration factor achieved using the various flow rate and TMP combinations was 126-fold (60% recovery), which is comparable to values previously reported by other workers for concentrating bacterial suspensions *(18, 26)*. Neither flow rate nor TMP had a significant ($P > 0.05$) effect on the levels of *Salmonella* recovered (Fig. 2). This may have been unexpected given that flow rate and TMP collectively defined the shear forces imposed on the cells, which, if excessive, results in membrane disruption *(26)*. It has been previously reported that high shear forces significantly decrease the recovery of yeasts cells by cross-flow filtration *(26)*. However, in the present study it was evident that *Salmonella* was less sensitive to the shear forces generated, thereby retaining viability during the filtration process. In practical terms it is desirable to have high flow rates to facilitate rapid filtration and low TMP to reduce the stresses imposed on the TFF, thereby extending the working lifetime of the filter *(38)*. Spent irrigation water contains solids derived from bean exudates, in addition to microbial biomass and constituents that could impact on the filtration efficacy of TFF. It was found that the solids content of spent irrigation water collected at different time periods during the sprouting period varied significantly ($P < 0.05$) (Fig. 3). Spent irrigation water collected shortly after the beans had been soaked in water for 24 h contained higher solids content than did samples collected toward the end of the sprouting period (Fig. 3). The changes in solids content can be attributed to

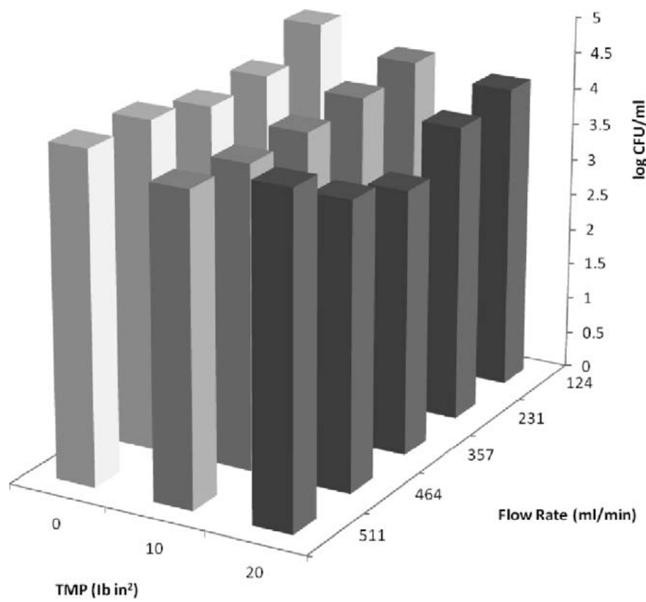

FIGURE 2. *Effect of TFF flow rate and TMP on the concentration of* Salmonella *from suspensions originally containing 2 log CFU/ml.* Salmonella *suspensions were prepared in saline and passed through the TFF unit at different TMP and flow rates. The cell density of* Salmonella *within the retentate following filtration was then determined and used to calculate the concentration factor.*

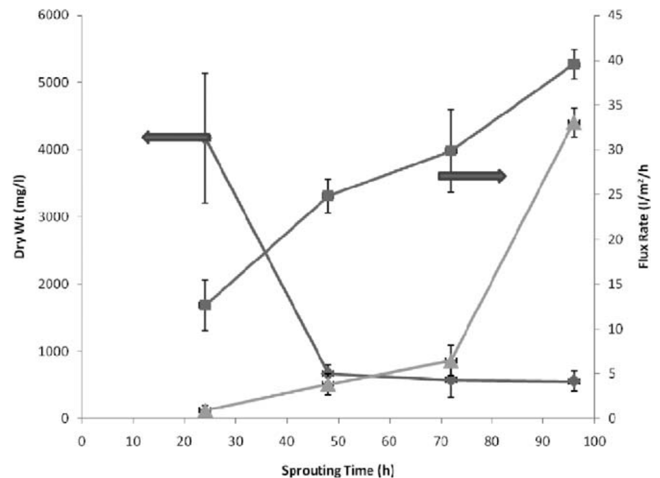

FIGURE 3. *Effect of solids content of spent mung bean irrigation water on the flux rate through the TFF unit. Mung bean batches (500 g) were sprouted for 96 h at 25°C and watered daily with 2 liters of irrigation water. The spent irrigation water was collected, and 100-ml samples were withdrawn for dry-weight analysis (◆). The remaining spent irrigation water was passed through TFF with an operating TMP of either 3.5 lb/in² (■) or 0.5 lb/in² (▲) and a flow rate of 0.51 liters/min.*

the release of exudates from the germinating beans that are maximal at the early stages of sprout development *(46).* Relevant to the present study, the high solids content of the spent irrigation water negatively affected the flux rate of TFF (Fig. 3). The effect of solids content on flux rates can be attributed to the accumulation of organic matter within the pores of the TFF hollow fiber that impedes the flow of water through the membrane *(2, 9, 29).*

The solids content of spent irrigation water collected from mung bean beds 48 to 96 h into the sprouting period was not significantly ($P > 0.05$) different. However, the flux rate observed for irrigation water collected at either 48 or 72 h was significantly ($P < 0.05$) lower than that of samples withdrawn at 96 h (Fig. 3). Such differences may be attributed to the composition of solids content. For example, polysaccharide materials result in agglomeration of particulates which would result in increased biofouling compared to low-molecular-weight constituents such as organic acids *(8, 19).* Regardless of this fact the results indicated that spent irrigation water collected at the end of the sprouting period resulted in less biofouling of the membrane and facilitated high filtration rates.

The reduced flux with increasing solids content could be compensated to a degree by increasing the TMP (Fig. 3). By using a higher TMP (3.5 lb/in^2), an increase in shear force is achieved that minimizes the accumulation of organic matter within the pores of the TFF hollow fiber, thereby maintaining high flux rates *(8).*

For optimal recovery of *Salmonella* it is desirable to collect samples when levels of the enteric pathogen within the sprouting mung bean bed are maximal. Therefore, trials were performed to determine the levels of *Salmonella* associated with spent irrigation water collected at different times during the sprouting period. To mimic the worst-case scenario, *Salmonella* was introduced at low levels (1.3 log CFU/g) into 500-g mung bean batches. The levels of *Salmonella* remained relatively constant during the initial 48 h into the sprouting period and increased thereafter, attaining levels of 2.5 ± 0.4 log CFU/ml at 96 h. The results were in agreement with those of Hora et al. *(15),* who also reported low *Salmonella* levels in spent irrigation water when the enteric pathogens were introduced at cell densities comparable to those used in the present study. The low level of *Salmonella* was unexpected given that other reports have demonstrated rapid growth of the pathogen on sprouting seeds *(17, 42).* However, it should be noted that in the majority of studies to date seeds were inoculated with a relatively high inoculum level (>4 log CFU/g) and also levels of the enteric pathogen were enumerated on sprouts as opposed to spent irrigation water.

The low *Salmonella* levels within spent irrigation water observed in the present study can be explained by the strong attachment of the human pathogen on sprouts *(7).* Consequently, *Salmonella* is more likely to be retained within the sprout bed than washed out during irrigation *(15).* A further possibility is the suppression of *Salmonella* growth by the antagonistic action of endogenous microflora *(10).* In the present study it was noted that the total aerobic count associated with spent irrigation water progressively increased during the sprouting period (Fig. 4). Therefore, it is likely that *Salmonella* introduced at low levels could not effectively compete in the early stages of the sprouting period.

It has been recommended that spent irrigation water should be sampled 48 h into the sprouting period *(1).* Based on the results obtained in this study, sampling early in the sprouting production would reduce the possibility of de-

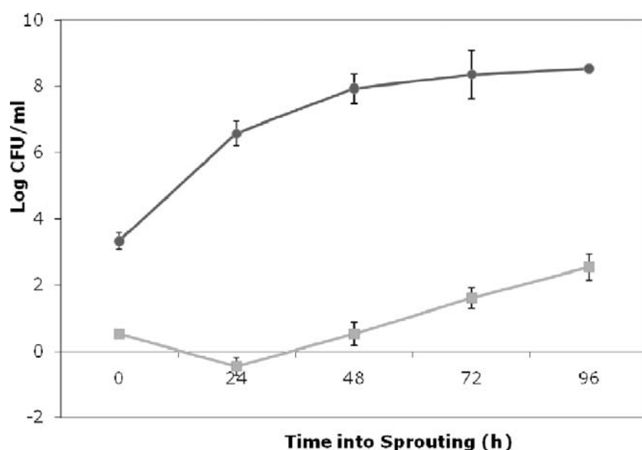

FIGURE 4. *Total aerobic count and* Salmonella *count within spent irrigation water derived from sprouting of mung bean batches at different times during sprouting. Batches (500 g) of mung beans were inoculated with* Salmonella *(1.3 log CFU/g). The beans were sprouted for 96 h at 25°C and irrigated daily with 2 liters of water. The spent water was collected, and* Salmonella *levels were determined.*

tecting *Salmonella* if present. However, by performing spent irrigation water screening at the end of the sprouting period, the levels of *Salmonella* are maximal and consequently more likely to be detected *(15).*

The recovery of *Salmonella* inoculated into spent irrigation water was evaluated. Here spent irrigation water was collected from sprouting mung bean beds 96 h into the sprout production and inoculated with defined levels of *Salmonella* by using the 7-log CFU/ml suspension (Table 1). The inoculated spent irrigation water was then concentrated using the optimized TFF. It was found that the cell density of pathogens could be increased 10- to 16-fold by using TFF (Table 1). The percent recovery of *Salmonella* in spent irrigation water was lower than that observed in saline (Fig. 2). In addition, it was noted that the percent recovery of *Salmonella* was significantly ($P < 0.05$) decreased when 2-log CFU/ml suspensions were processed compared with 4.5 log CFU/ml (Table 1). The lower recovery yields with dilute *Salmonella* suspensions could have been due to cells binding to the membrane surface, a process that occurs during the filtration process *(26).*

Clean-in-place of TFF unit. Although the TTF cartridges used in this study were only intended for single use, it can be envisaged that in commercial practice the filter would perform multiple runs. This necessitates developing clean-in-place protocols that can ensure inactivation and removal of residual microbial cells without compromising the integrity of the filter membrane. From the sanitizers tested it was evident that sodium hydroxide proved least effective with *Salmonella* being recovered following the clean-in-place cycle. In contrast, both hypochlorite and acidified sodium chlorite were effective at sanitizing the TFF unit. However, applying acidified sodium chlorite caused accumulation of white crystals within the filter and hence negatively impacted on performance. Consequently, sodium hypochlorite was used in subsequent studies for sanitizing

TABLE 1. *Increased concentration and percent recovery after concentrating 2 liters of spent irrigation water containing* Salmonella[a]

Salmonella count (log CFU/ml)		
Initial suspension	Retentate (CF)[b]	% recovery[c]
4.5	5.7 ± 0.58 (15.85)	82 ± 8
2.0	3.0 ± 0.13 (10.00)	55 ± 8

[a] TMP, 3.5 lb/in^2; flow rate, 511 ml/min.
[b] CF, concentration factor (calculated as CFU in retentate/CFU in initial suspension).
[c] % recovery = (total CFU in retentate/total CFU in initial suspension) × 100.

the TFF between pressure runs. In general the filters could be used to process eight times before failing (i.e., recovery of *Salmonella* from the permeate). No progressive decrease in filtration performance (i.e., capture yield or flux rate) was observed between TFF runs. However, when membrane failure occurred there was an abrupt loss of TMP, which coincided with *Salmonella* being recovered in the filtrate.

Flowthrough amperometric immunosensor design and optimization. A flowthrough immunoassay was designed based on passing the retentate derived from the TFF step over a membrane modified with anti-*Salmonella* antibodies. In addition to acting as a solid support, the membrane also functioned as a permselective membrane to prevent biofouling of the electrode that was used to detect the hydrogen peroxide enzymatic product of the glucose oxidase antibody conjugate. The three different base membrane types evaluated were cellulose acetate, plasticized PVC, and PES. All three polymers are widely used in biosensor devices due to biocompatibility or permselectivity characteristics *(3, 37).* The PES membrane was too brittle and could not be overlaid over the electrode without breaking, and PVC was readily passivated by proteins. However, cellulose acetate membranes were both biocompatible and permeable to hydrogen peroxide. Biocompatibility was evaluated by determining the response to hydrogen peroxide in the presence and absence of BSA or heat-killed cells. Although the hydrogen peroxide amperometric response was lower in the presence of BSA or heat-killed cells, this was insignificantly ($P > 0.05$) different compared with when dose-response curve assays were performed with KCl alone (Fig. 5).

Immobilization of antibodies onto cellulose acetate membranes. Anti-*Salmonella* antibody was immobilized onto the surface of gold-sputtered cellulose acetate membranes via thiol coupling. Verification of antibody immobilization was performed by reacting the modified cellulose acetate membrane with diluted *Salmonella* suspensions (6 log CFU/ml). After incubation at room temperature for 40 min the membrane was rinsed to remove nonattached *Salmonella* prior to viewing under scanning electron microscope. *Salmonella* cells were observed on antibody-modified films, although negligible numbers were encountered on nonmodified membranes (Fig. 6). The results confirm

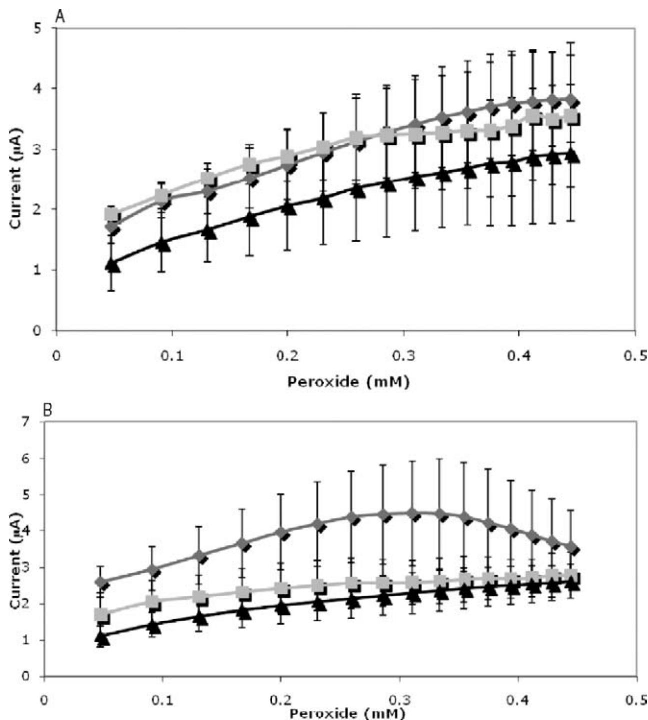

FIGURE 5. *Response of a cellulose acetate modified platinum electrode to hydrogen peroxide in the presence of 0.1% BSA (A) or heat-killed (4 log CFU/ml)* Salmonella *(B). Step calibration curve assays were performed by addition of 1 mM hydrogen peroxide (◆) to a polarized (0.65 V versus Ag/AgCl) platinum electrode modified with a cellulose acetate membrane. The dose-response to hydrogen peroxide was repeated in the presence of BSA or heat-killed cells (■), using the same cellulose acetate membrane. Finally, the dose-response of the cellulose acetate membrane to hydrogen peroxide alone was assessed to determine the degree of biofouling (▲). Data points represent the average of three different cellulose acetate membranes.*

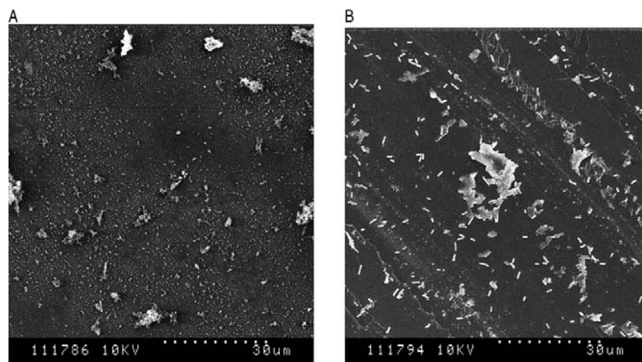

FIGURE 6. *Scanning electron micrographs of cellulose acetate membranes without (A) and with (B) immobilized capture antibody. A thin gold layer was deposited onto the surface of cellulose acetate membranes upon which capture antibody was immobilized. The membrane was allowed to react with a* Salmonella *suspension and subsequently was rinsed to remove unbound cells. The control was prepared in the same way except that no capture antibody was immobilized on the membrane surface.*

that the antibody-modified cellulose acetate resulted in specific binding of *Salmonella* with minimal nonspecific binding.

The anti-*Salmonella*–modified cellulose acetate membrane was integrated into a flow cell, and capture efficiency was determined. Here, different *Salmonella* serovar suspensions (100 ml) were flowed (0.89 ml/min) over the antibody layer, and changes in levels of the enteric pathogen remaining were used to calculate the capture efficiency (Table 2). The capture efficiency of anti-*Salmonella*–modified cellulose acetate membranes ranged from 64 to 95% (Table 2). The similar capture efficiencies obtained with the different serovars confirm that the sensor could be used to capture the different *Salmonella* types tested.

The level of *Salmonella* captured is comparable to that obtained with immune columns *(5)* and paramagnetic beads *(40),* both of which have reported efficiencies of 95%. In the present study the high capture efficiencies could be attributed to the high affinity of the anti-*Salmonella* antibodies. However, it should be noted that the nonspecific binding (i.e., films containing no antibodies) was relatively high (12 to 36%) (Table 2). The nonspecific binding was likely caused by the attachment of cells to the tubing and reaction cell. However, the capture of *Salmonella* was consistently higher, with antibody-modified films indicating that the majority of binding was specific and not due to passive attachment.

The captured cells were detected by allowing *Salmonella* to react with a secondary rabbit anti-*Salmonella* biotin-labeled antibody followed by avidin-tagged glucose oxidase. Glucose enzyme substrate was then added, and the hydrogen peroxide product was detected amperometrically at the underlying platinum electrode. The assay was optimized in terms of assay time, temperature, concentration of antibody, and glucose oxidase. The optimal sensor response was found by allowing 6 µl (2 mg/ml) of biotin-labeled antibody to react with 120 µg of glucose oxidase at 23°C for 40 min (results not shown).

Evaluation of the integrated TFF and amperometric immunosensor to detect *Salmonella* in spent irrigation water. Spent irrigation water (2 liters) was collected from mung bean beds initially inoculated with different levels of *Salmonella* (1.3 to 3.3 log CFU/g) (Table 3). The level of

TABLE 2. *Capture of different* Salmonella *serovars on anti-*Salmonella *modified and nonmodified cellulose acetate membranes*[a]

Salmonella serovar	log CFU of *Salmonella*/ml (% capture) on[b]: Anti-*Salmonella* modified CA[c]	Control CA
Heidelberg	2.17 ± 0.35 (90) A	2.96 ± 0.17 (36) B
Senftenberg	1.88 ± 0.39 (95) A	3.10 ± 0.31 (12) B
Montevideo	2.58 ± 0.23 (73) A	3.03 ± 0.32 (25) B
Newport	2.12 ± 0.20 (91) A	3.06 ± 0.29 (20) B
Meleagridis	2.71 ± 0.21 (64) A	3.04 ± 0.31 (24) B
Oranienburg	2.39 ± 0.32 (83) A	2.99 ± 0.32 (32) B

[a] Values within rows followed by the same letter are not significantly different.
[b] % capture = (CFU remaining in suspension/total initial CFU) × 100.
[c] CA, cellulose acetate membrane.

TABLE 3. *Concentration and detection of* Salmonella *from spent irrigation water derived from 96-h sprouting mung bean beds initially inoculated with different levels of the pathogens*

Initial loading (log CFU/g)	*Salmonella* count in: Spent irrigation water (log CFU/ml)	*Salmonella* count in: Retentate (log CFU/ml) (CF)[b]	Sensor response (nA)[a]
0	0	0	32.6 ± 19.82 A
1.3	2.43 ± 0.39	3.0 ± 0.13 (4)	342.1 ± 135.04 B
1.9	3.74 ± 0.36	5.7 ± 0.58 (91)	364.9 ± 137.10 B
3.3	4.17 ± 0.46	7.47 ± 0.46 (3,715)	668.4 ± 71.91 C

[a] Means followed by the same letter are not significantly ($P < 0.05$) different.
[b] CF, concentration factor (calculated as CFU in retentate/CFU in initial suspension).

Salmonella recovered from the spent irrigation water at the end of the 96-h sprouting period was dependent on the initial inoculation introduced onto the mung beans (Table 3). The same finding has been reported for the growth of *Salmonella* introduced at different levels within sprouting alfalfa sprouts *(24).*

The concentration factor when the spent irrigation water was passed through the TFF was found to be dependent on the *Salmonella* concentration in the sample (Table 3). Low levels of *Salmonella* in the spent irrigation water resulted in the lowest concentration factor, which was again possibly due to binding of cells to the filtration unit. It was noted that the concentration factors were higher than when *Salmonella* was directly inoculated into spent irrigation water (Table 1). This may have been attributed to the physiological state of the cells, which enhanced tolerance to shear effects and/or reduced attachment to the filter membrane.

The response of the amperometric immunosensor correlated ($r^2 = 0.91$) with the *Salmonella* levels within the retentate. However, it is unlikely that the sensor could be used to quantify *Salmonella* organisms present within the sprouting mung bean bed given the variability in recovery yield by using TFF and capture efficiency of the antibody-modified membranes. The lack of quantitative measurement should not be considered a weakness of the sensor given that any *Salmonella* detected would be considered a hazard. In this respect the lower detection limit is of greater significance, and as observed, the sensor was able to detect *Salmonella* concentrations on the order of 2 log CFU/ml following TFF. The lower detection limit in the flowthrough immune sensor in isolation is comparable to other reported flowthrough immune sensors, which typically can detect levels on the order of 3 to 4 log CFU/ml *(6, 31).*

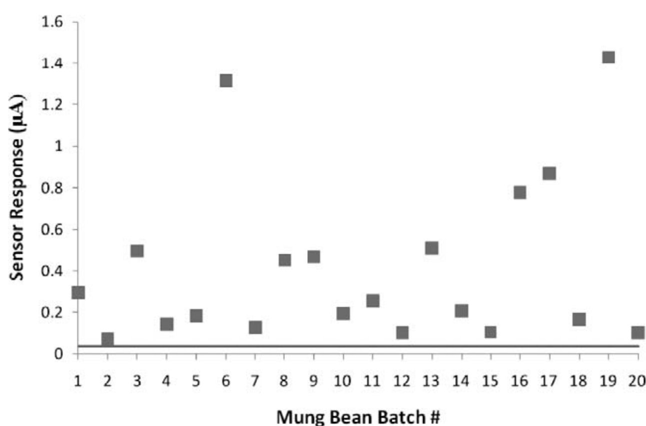

FIGURE 7. *Verification of integrated TFF and immune sensor to detect* Salmonella *in spent irrigation water collected from mung bean beds 96 h into the sprouting process.* Salmonella *was inoculated into mung bean batches (1.3 log CFU/g) and sprouted for 96 h with daily irrigation with 2 liters of water. At the end of the sprouting period, 2 liters of spent irrigation water was collected and concentrated using TFF. The retentate was then applied to the amperometric ELISA. The sensor responses for individual samples were plotted (■) and compared with the average responses obtained for samples derived from noninoculated controls (—).*

To test the reliability of the integrated TFF sensor system, 20 trials were performed whereby mung bean beds were inoculated with 1.3 log CFU of *Salmonella* per g and allowed to sprout for 96 h (Fig. 7). The spent irrigation water (2 liters) was passed through the TFF system, and retentate was transferred to the flowthrough immune sensor. Each TFF filter cartridge was reused seven times with the clean-in-place hypochlorite decontamination step as previously described. A fresh antibody-modified cellulose acetate membrane was used on each occasion.

The sensor response was found to be variable although consistently higher than the baseline (spent irrigation water derived from noninoculated mung bean beds). *Salmonella* was not detected in spent irrigation water taken from the control (noninoculated) mung bean batches, confirming that no false positives were generated by the sensor. Inoculated mung beans in Trial 2 gave a low response (Fig. 7), which was not significantly different from the baseline value and hence can be regarded as a false negative given that the retentate sample tested positive for *Salmonella* upon enrichment. Several samples resulted in high sensor responses, which may be related to variation in the fabricated antibody-modified membrane or levels of *Salmonella* within the retentate. However, regardless of this fact the results demonstrated the consistency of the integrated system to detect *Salmonella* within spent irrigation water. Furthermore, *Salmonella* captured on the membrane could be isolated by enrichment and subsequent plating onto selective media, thereby enabling conformational tests to be performed in addition to epidemiology investigations.

In conclusion, the study has provided proof-of-princi-

ple for an integrated device to screen spent irrigation water derived from sprouting mung bean beds. The method is highly sensitive and can detect *Salmonella* in spent irrigation water within 4 h. Due to the rapid analysis time it is possible to take samples at the end of the sprouting process, when *Salmonella* numbers are high. The system can be potentially automated, and TFF cartridges can be reused, thereby making the system a commercially viable alternative to current testing procedures. However, further studies are needed to verify the device performance in capturing and detecting a wider range of *Salmonella* serovars that may be encountered in sprouted seed production.

ACKNOWLEDGMENTS

The authors thank the commercial sprout grower for donating mung beans used in the study and Ontario Ministry of Agriculture and Food (OMAFRA) Food Safety Research and Innovation Program (project FS050633) for their financial support.

REFERENCES

1. Anonymous. 1999. Microbiological safety evaluations and recommendations on sprouted seeds. National Advisory Committee on Microbiological Criteria for Foods. *Int. J. Food Microbiol.* 52:123–153.
2. Baruah, G. L., A. Nayak, E. Winkelman, and G. Belfort. 2006. Purification of monoclonal antibodies derived from transgenic goat milk by ultrafiltration. *Biotechnol. Bioeng.* 93:747–754.
3. Benmakroha, Y., I. Christie, M. Desai, and P. Vadgama. 1996. Poly(vinyl chloride), polysulfone and sulfonated polyether-ether sulfone composite membranes for glucose and hydrogen peroxide perm-selectivity in amperometric biosensors. *Analyst* 12:521–526.
4. Bilitewski, U., M. Genrich, S. Kadow, and G. Mersal. 2003. Biochemical analysis with microfluidic systems. *Anal. Bioanal. Chem.* 377:556–569.
5. Brewster, J. D. 2003. Isolation and concentration of Salmonellae with an immunoaffinity column. *J. Microbiol. Methods* 55:287–293.
6. Brooks, J. L., B. Mirhabibollahi, and R. G. Kroll. 1992. Experimental enzyme-linked amperometric immunosensors for the detection of salmonellas in foods. *J. Appl. Bacteriol.* 73:189–196.
7. Charkowski, A. O., J. D. Barak, C. Z. Sarreal, and R. E. Mandrell. 2002. Differences in growth of *Salmonella enterica* and *Escherichia coli* O157:H7 on alfalfa sprouts. *Appl. Environ. Microbiol.* 68:3114–3120.
8. Colton, R. H., I. Pahl, L. E. Ottaviano, T. Bodeutsch, and F. Meyeroltmanns. 2002. Study of protein adsorption effects on crossflow filtration using BSA and milk protein PDA. *J. Pharm. Sci. Technol.* 56:20–30.
9. Daubert, I., M. Mercier-Bonin, C. Maranges, G. Goma, C. Fonade, and C. Lafforgue. 2003. Why and how membrane bioreactors with unsteady filtration conditions can improve the efficiency of biological processes. *Ann. N. Y. Acad. Sci.* 984:420–435.
10. Fett, W. E. 2006. Inhibition of *Salmonella enterica* by plant-associated pseudomonads in vitro and on sprouting alfalfa seed. *J. Food Prot.* 69:719–728.
11. Fu, T.-J., K. Reineke, T. Krupinski, O. VanPelt, N. Maks-Warren, B. Parisi, and P. Slade. 2005. Integrating tangential flow filtration with rapid tests for detection of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* in large volumes of spent sprout irrigation water. IAFP Annual Meeting, 13 to 17 August 2005, Baltimore, Md.
12. Fu, T. J., D. Stewart, K. Reineke, J. Ulaszek, J. Schlesser, and M. Tortorello. 2001. Use of spent irrigation water for microbiological analysis of alfalfa sprouts. *J. Food Prot.* 64:802–806.
13. Gamliel, H. 1985. Optimum fixation conditions may allow air drying of soft biological specimens with minimum cell shrinkage and maximum preservation of surface features. *Scan. Electron Microsc.* 4:1649–1664.
14. Genovesi, C. S. 1983. Several uses for tangential-flow filtration in the pharmaceutical industry. *J. Parenter. Sci. Technol.* 37:81–86.
15. Hora, R., M. Kumar, L. Garcia, B. Schumacher, J. Odumeru, and K. Warriner. 2005. Spatial distribution of *Salmonella, Escherichia coli* O157:H7, and other bacterial populations in commercial and laboratory-scale sprouting mung bean beds. *J. Food Prot.* 68:2510–2518.
16. Hora, R., K. Warriner, B. J. Shelp, and M. W. Griffiths. 2005. Internalization of *Escherichia coli* O157:H7 following biological and mechanical disruption of growing spinach plants. *J. Food Prot.* 68:2506–2509.
17. Howard, M. B., and S. W. Hutcheson. 2003. Growth dynamics of *Salmonella enterica* strains on alfalfa sprouts and in waste seed irrigation water. *Appl. Environ. Microbiol.* 69:548–553.
18. Jiang, S. C., J. M. Thurmond, S. L. Pichard, and J. H. Paul. 1992. Concentration of microbial populations from aquatic environments by vortex flow filtration. *Mar. Ecol. Prog.* 80:101–107.
19. Johns, M. R., and E. Noor. 1991. Recovery and purification of polysaccharides from microbial broth. *Aust. J. Biotechnol.* 5:73–77.
20. Johnson, R. N., and J. R. Baker. 1998. Accuracy of devices used for self-monitoring of blood glucose *Ann. Clin Biochem.* 35:68–74.
21. Johnston, L. M., D. Elhanafi, M. Drake, and L. A. Jaykus. 2005. A simple method for the direct detection of *Salmonella* and *Escherichia coli* O157:H7 from raw alfalfa sprouts and spent irrigation water using PCR. *J. Food Prot.* 68:2256–2263.
22. Kramer, M. F., and D. V. Lim. 2004. A rapid and automated fiber optic-based biosensor assay for the detection of *Salmonella* in spent irrigation water used in the sprouting of sprout seeds. *J. Food Prot.* 67:46–52.
23. Liao, C. H. 2008. Growth of *Salmonella* on sprouting alfalfa seeds as affected by the inoculum size, native microbial load and *Pseudomonas fluorescens* 2-79. *Lett. Appl. Microbiol.* 46:232–236.
24. Liu, B., and D. W. Schaffner. 2007. Quantitative analysis of the growth of *Salmonella* Stanley during alfalfa sprouting and evaluation of *Enterobacter aerogenes* as its surrogate. *J. Food Prot.* 70:316–322.
25. Marquette, C. A., and L. J. Blum. 2006. State of the art and recent advances in immunoanalytical systems. *Biosens. Bioelectron.* 211:424–433.
26. Mawson, A. J. 1993. Crossflow microfiltration of cell suspensions. *Australas. Biotechnol.* 3:348–352.
27. Mohle-Boetani, J. C., J. Farrar, P. Bradley, J. D. Barak, M. Miller, M. R. Mandrell, P. Mead, W. E. Keene, K. Cummings, S. Abbott, and S. B. Werner. 2008. *Salmonella* infections associated with mung bean sprouts: epidemiological and environmental investigations. *Epidemiol. Infect.* 2008:1–10.
28. Ordonez, S. S., and E. Fabregas. 2007. New antibodies immobilization system into a graphite-polysulfone membrane for amperometric immunosensors. *Biosens. Bioelectron.* 22:965–972.
29. Phelps, T. J., A. V. Palumbo, and B. L. Bischoff. 2008. Micron-poresized metallic filter tube membranes for filtration of particulates and water purification. *J. Microbiol. Methods* 74:10–16.
30. Reddy, S. M., S. P. Higson, I. M. Christie, and P. M. Vadgama. 1994. Selective membranes for the construction and optimization of an amperometric oxalate enzyme electrode. *Analyst* 119:949–952.
31. Ricci, F., G. Volpe, L. Micheli, and G. Palleschi. 2007. A review on novel developments and applications of immunosensors in food analysis. *Anal. Chim. Acta* 605:111–129.
32. Stewart, D. S., K. F. Reineke, and M. L. Tortorello. 2002. Comparison of assurance gold *Salmonella* EIA, BAX for screening/*Salmonella,* and GENE-TRAK Salmonella DLP rapid assays for detection of *Salmonella* in alfalfa sprouts and sprout irrigation water. *J. AOAC Int.* 85:395–403.
33. Stewart, J. R., R. D. Ellender, J. A. Gooch, S. Jiang, S. P. Myoda, and S. B. Weisberg. 2003. Recommendations for microbial source tracking: lessons from a methods comparison study. *J. Water Health* 1:225–231.
34. Taormina, P. J., and L. R. Beuchat. 1999. Behavior of enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa sprouts during the sprouting process as influenced by treatments with various chemicals. *J. Food Prot.* 62:850–856.
35. Tiefenauer, L. X., S. Kossek, C. Padeste, and P. Thiebaud. 1997.

Towards an amperometric immunosensor devices. *Biosens. Bioelectron.* 12:213–223.

36. Urraca, J. L., E. Benito-Pena, C. Perez-Conde, M. C. Moreno-Bondi, and J. J. Pestka. 2005. Analysis of zearalenone in cereal and Swine feed samples using an automated flow-through immunosensor. *J. Agric. Food Chem.* 53:3338–3344.
37. Vadgama, P. 2007. Sensor biocompatibility: final frontier in bioanalytical measurement. *Analyst* 13:2495.
38. van Reis, R., S. Gadam, L. N. Frautschy, S. Orlando, E. M. Goodrich, S. Saksena, R. Kuriyel, C. M. Simpson, S. Pearl, and A. L. Zydney. 1997. High performance tangential flow filtration. *Biotechnol. Bioeng.* 56:71–82.
39. van Reis, R., and A. Zydney. 2001. Membrane separations in biotechnology. *Curr. Opin. Biotechnol.* 12:208–211.
40. Varshney, M., L. Yang, X. L. Su, and Y. Li. 2005. Magnetic nanoparticle-antibody conjugates for the separation of *Escherichia coli* O157:H7 in ground beef. *J. Food Prot.* 68:1804–1811.
41. Wang, S., X. Ji, and Z. Yuan. 1995. Study of cellulose acetate membrane-based glucose biosensors. *Chin. J. Biotechnol.* 11:199–205.
42. Warriner, K., S. Spaniolas, M. Dickinson, C. Wright, and W. M. Waites. 2003. Internalization of bioluminescent *Escherichia coli* and *Salmonella* Montevideo in growing bean sprouts. *J. Appl. Microbiol.* 95:719–727.
43. Winthrop, K. L., M. S. Palumbo, and J. A. Farrar. 2003. Alfalfa sprouts and *Salmonella* Kottbus infection: a multistate outbreak following inadequate seed disinfection with heat and chlorine. *J. Food Prot.* 66:13–17.
44. Yamazaki, M., K. Mitamura, and M. Ichikawal. 2004. Evaluation of flow-through immunoassay for rapid detection of influenza A and B viruses. *Kansenshogaku Zasshi* 78:865–871.
45. Zeravik, J., T. Ruzgas, and M. Franek. 2003. A highly sensitive flow-through amperometric immunosensor based on the Peroxidase chip and enzyme-channeling principle. *Biosens. Bioelectron.* 18:1321–1327.
46. Zheng, S. H., and M. Kawabata. 2000. Exudation of amino acids and sugars from imbibing seeds of several leguminous crops. *J. Crop Sci.* 69:380–384.