Reduction of Microorganisms via Green Tea Bioreactor Treatment and Rapid Microbial Determination with Quantum Dots Labeled Method

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Abstract:

Cadmium–telluride (CdTe) quantum dots as a fluorescence label, and a rapid, simple, and sensitive quantum dots (QDs) labeled method was employed to detect the total count of bacteria and fungi, using 1-Ethyl-3-(3-dimethyllaminopropyl)-carbodiimide (EDC) as a crosslinker. CdTe quantum dots could successfully be conjugated with the representive bacteria Escherichia coli, mold Aspergillus niger and yeast Saccharomyces cerevisiae. This method could count bacteria and fungi with content of 102 CFU/mL. The determination of total count of bacteria and fungi with fluorescence method for three samples of tap water, river water and lake water were similar to the standard plate count method. The green tea powder bioreactor was constructed to treat the three samples, and the QDs labeled method was used to test the reduced bacteria and fungi, and the results revealed that this green tea powder bioreactor could effectively reduce the amount of microorganisms of the three samples.

Key words:

Quantum Dots; Green Tea Powder; Bioreactor; Microorganisms' Reduction

Introduction

Bacteria, mold and yeast are common pathogens that can cause infectious diseases. The conventional methods were based on plate count; however, it could not perfectly reach the requirements of microbial detection because of slow detection speed (Kubitschek, 1990). Recently, many novel methods were applied in microbial detection, such as enzyme linked immunosorbent assay (ELISA) (Thornton et al, 2010; Blay et al, 2004; Iwahori et al, 2000), Taqman real-time polymerase chain reaction (PCR) (Dreier et al, 2007; Studer et al, 2008; Takahashi et al, 2005) and flow cytometry detection (Chang et al, 2004; Hussein et al, 2002; Pianetti et al, 2008), InfraRed Epifluorescence Microscopic (TIREM) (Jiao et al, 2006), DNA microarray (Wang et al, 2007), and immunomagnetic separation (IMS) methods (Ochoa and Harrington, 2005). However, although so many methods were applied in microbial detection, there were some drawbacks hindering their widely application in practical fields, like complex procedure, highly cost for apparatus and others, time-consuming or low sensitivity.

Recently, fluorescence analysis was developed as a rapid, technically simple, and efficient detection method of microbe to overcome the disadvantages of traditional methods (Coulon et al, 2010; Su and Li, 2005; Vinayaka and Thakur, 2010; Yang and Li, 2006; Zhao et al, 2009), some researchers have demonstrated fluorescence measurement methods for rapid detection of bacterial count by using quantum dots (QDs) as a fluorescence marker (Fu et al, 2009). Quantum dots are bright, photostable fluorophores semiconductor nanocrystals, which possess broad excitation spectrum but narrow Gaussian emission at wavelengths controllable by the size of the material. Due to their potential applications in the fields of chemistry and biomedical as fluorescence markers and luminescent biological labels, much current research focuses on understanding their behavior in liquid environments (Bruchez et al, 1998; Chan and Nie, 1998; Larson et al, 2003; Xue et al, 2009).

Green tea and its effective components such as tea

polyphenols (TP) were proved to have a broad antimicrobial spectrum, which could inhibit many species of microbe, such as Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus and Candida species (Almajano et al, 2008; An et al, 2004; Hamilton-Miller, 1995; He et al, 2006; Park et al, 2006; Si et al, 2006). Thus, using green tea as an antimicrobial agent for a bioreactor is reasonable.

In this study, we set up a rapid, simple, and efficient fluorescence method for bacterial and fungul counting was developed using CdTe quantum dots (QDs) as fluorescence marker, and Escherichia coli (bacteria), Aspergillus niger (mold) and Saccharomyces cerevisiae (yeast) were used as representative strains. Moreover, a green tea powder bioreactor was constructed to reduce the amount of bacteria and fungi in three different samples of lake water, river water and tap water, and the quantum dots CdTe labeled method was appiled to determine the microbial reduction level.

Materials and Methods

Materials

Cadmium chloride, sodium borohydride, tellurium powder and sodium hydroxide (Sinopharm Reagent Co.Ltd., Shanghai, China) were used for the preparation of CdTe-QDs. Thioglycolic acid (TGA, Sigma-Aldrich, USA) was used as a stabilizer of CdTe-QDs.

1-Ethyl-3-(3-dimethyllaminopropyl)-carbodiimide (EDC, Sigma-Aldrich, USA) was used as coupling reagent. Yeast Extract, Peptone, Glucose were used as medium of microbe. All reagents were of analytical grade.

Culture of Three Representative Strains

All the culture collections were purchased from Shanghai Bioplus Biotechnology Co., Ltd. Escherichia coli (collection numbers: ATCC 2601; Catalogue: 0698), Aspergillus niger (collection numbers: ATCC 16888; Catalogue: 0245) and Saccharomyces cerevisiae (collection numbers: ATCC 4157; Catalogue 0433) as representative strains were cultured respectively. E.coli grown in LB culture medium for 12 hours and S. cerevisiae grown in YDP culture medium for 72 hours. Aspergillus niger was cultured in PDA agar medium for 5-6 days (till the mature spore come out) and then 5ml the aseptic physiological saline was added in the test tube. Loop-carrier was used to scrape the spore, and then the mixed liquor was gone through aseptic absorbent cotton to obtain the spore fluid.

Quantum Dots (Qds) Coupling With Three Strains

Water-soluble CdTe QDs solution was prepared as reported (Wang et al, 2009). The suspension of three microbial cells (E.coli, A.niger spore and S.cerevisiae) were centrifuged at 4500r/min for 10min, and the supernatant was discarded. Then, these three representative microbial cells were resuspended in 10mL PBS. The CdTe quantum dots were conjugated to them with EDC as coupling reagent. For measuring the best condition of water-soluble CdTe quantum dots coupled with the microbial cells, 50–500μL (1.0mg/mL) CdTe QDs and 100μL 0.1mg/mL EDC were reacted together with these microbial cell for 10–60 min at 37 ◦C, and then ultra-filtration membrane (0.22μm) was used to purify the mixtures and the excess CdTe QDs was washed away.

Fluorescence Microscopy and Fluorescence Intensity Determination

Three kinds of the quantum dots-cell suspension were observed under a fluorescence microscope (Leica, DMI 4000B, Germany) and fluorescence images were photographed. The fluorescence intensity of different QDs coupling representative strains including E.coli (represented bacteria), mixed with A.niger spore and S.cerevisiae (represented fungi) was determined with a fluorescence spectrophotometer (Hitachi, F-2500, Japan).

Standard and Qds Coupling Methods Testing Of Samples' Microorganism Quantity

Lake, river, and tap water were taken from Dianshui Lake, Suzhou River and room tap water in Shanghai, China, respectively. Samples were first passed through filter to remove impurities and filtration. Then, 0.8 μm filter membrane was applied to depart and determine the samples' total amount of fungi and bacteria. Each sample was tested both in QDs coupling method and standard plate count to compare the two methods in order to confirm the accuracy of the QDs coupling methods.

Determination the MIC of Representative Microbe

Different mass (0.05-0.5g) of green tea powder was added in 20ml Agar medium of Escherichia coli (LB), Aspergillus niger (PDA) and Saccharomyces cerevisiae(YDP), then 1ml log phase representatives

strains fluid were spreaded on flat plates. MICs were determined until plates had no colonies.

Preparation of the Green Tea Bioreactor and the Water Samples Treatment

A reasonable green tea reactor was designed and the water samples were passed through the bioreactor. The QDs methods mentioned in preceding part were used to determinate the total amount of the bacteria in the treated samples. All the experiments were taken under room temperature and the samples' nature pH.

Results

Fluorescence Microscopy

Bright field and fluorescence images of three representative strains were shown in Fig.1 and Fig.2. As shown, bright field and fluorescence images were observed under a fluorescent microscope. The results showed that the CdTe QDs and the surface of E. coli, A. niger and S.cerevisiae could effectively conjugated by EDC.

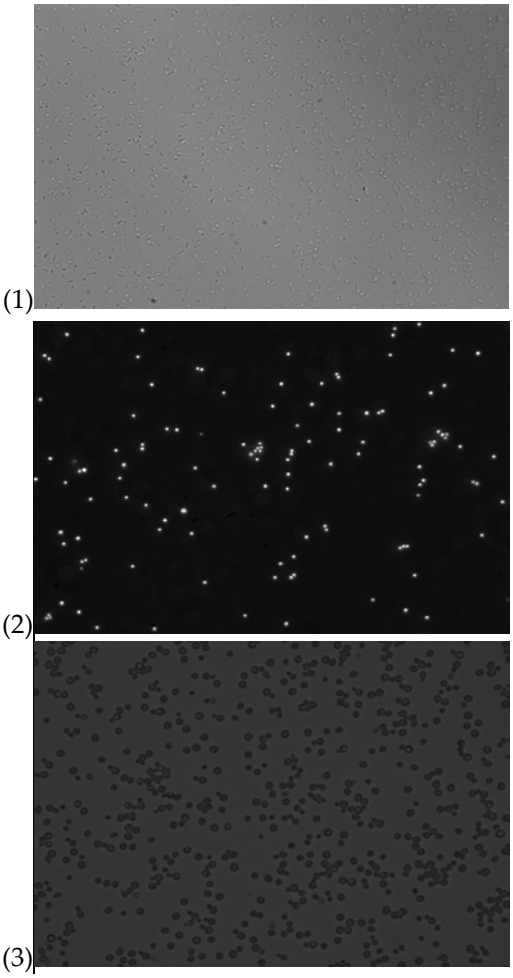

FIG.1 BRIGHT FIELD IMAGES OF THREE REPRESENTATIVE STRAINS (1-E. COLI, 2-A. NIGER SPORE, 3-S. CEREVISIAE).

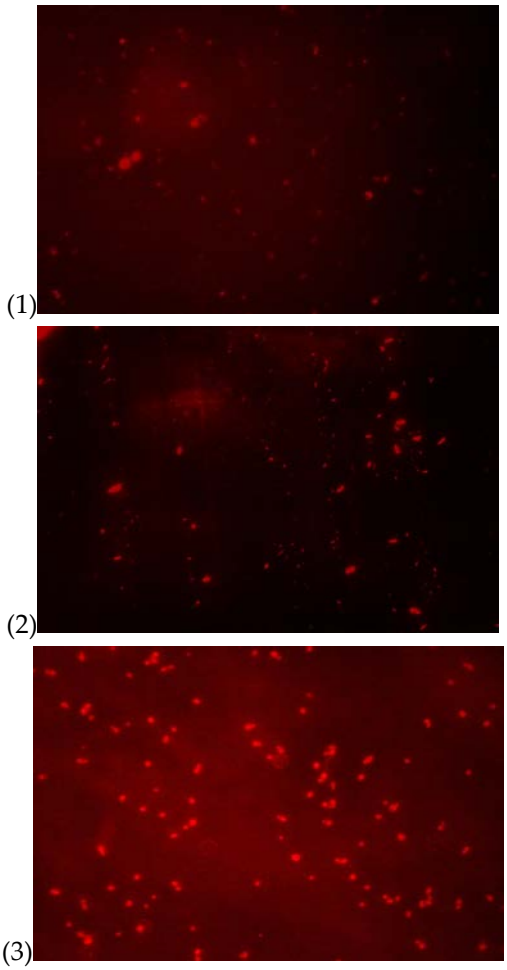

FIG. 2 FLUORESCENT MICROSCOPY OF THREE QUANTUM DOTS MARKED REPRESENTATIVE STRAINS (1-E. COLI, 2-A. NIGER SPORE, 3-S. CEREVISIAE)

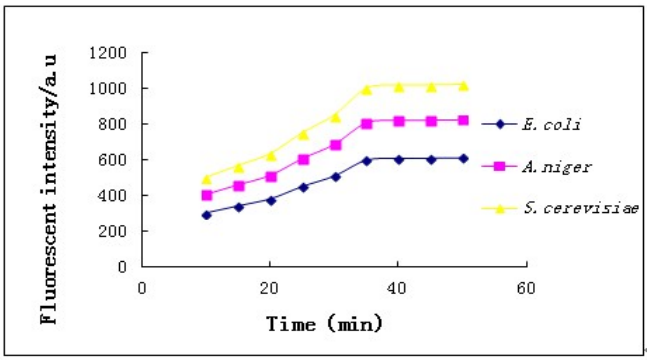

FIG. 3 EFFECT OF CDTE-MICROBE COMBINATION TIME UPON THE FLUORESCENCE INTENSITY

Optimization of the Condition of Cdte Quantum Dots Coupling With Microbe Cells

50-500 μL (1.0mg/mL) CdTe QDs and E. coli, A. niger spore and S. cerevisiae were combined for 60min respectively, to determine the best combination time of CdTe QDs coupled with these microbe cells. The fluorescence intensity of different combination time was obtained at 490 nm. As shown in Fig. 3, in the first 35 min, fluorescence intensity of E. coli, A. niger spore

and S. cerevisiae increase d with combination time, but the fluorescence intensity rarely increased in the later 25 min after addition of the CdTe QDs solution to the three representative strains. Thus 35min was chosen as the favorable combination time of CdTe QDs coupled with microbe cells in this study.

The best CdTe QDs concentrations of CdTe QDs coupled with representative strains were determined using 50-500 μL (1.0mg/mL) CdTe QDs combined together with E. coli, A. niger spore and S. cerevisiae, respectively, for 35 min. As shown in Fig.4, fluorescence intensity of E. coli, A. niger spore and S. cerevisiae coupled with CdTe QDs solution increased with CdTe QDs concentrations from 50 μL to 500 μL, but CdTe QDs coupled with microbe cells was limited with QDs concentrations and did not increase until CdTe QDs concentrations achieved 250 μL. Thus, the minimum concentration of the CdTe QDs was 250μL.

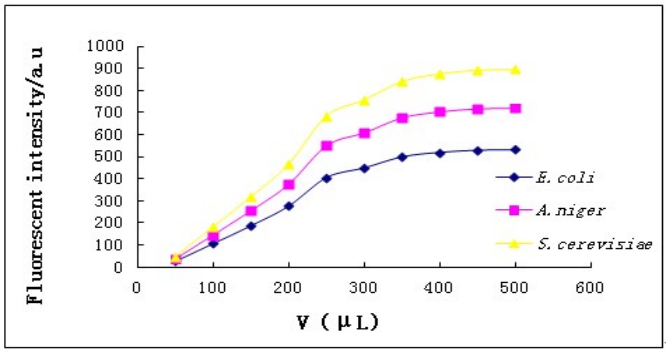

FIG.4 EFFECT OF CDTE QDS CONCENTRATIONS ON THE FLUORESCENCE INTENSITY OF THE QDS BIND ON E. COLI, A. NIGER SPORE AND S. CEREVISIAE IN LOG PHASE.

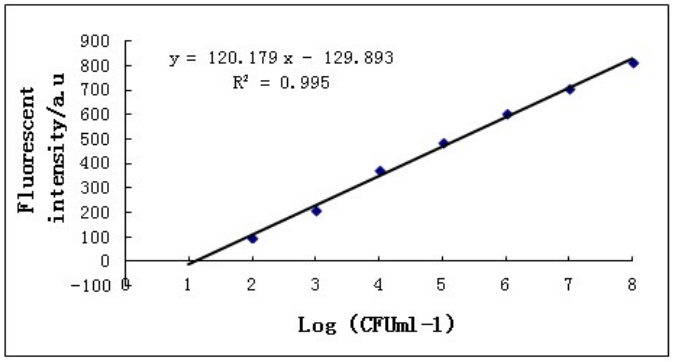

FIG.5 VARIATION IN DIFFERENTIAL FLUORESCENCE INTENSITY WITH TOTAL COUNT OF E.COLI (REPRESENTED BACTERIA)

Fluorescence Detection of Total Count of Bacteria and Fungi

The fluorescence intensity of 10^1–10^7 CFU/mL total count of bacteria and fungi was shown in Fig.5 and Fig.6. The fluorescence intensity increased with increasing cell count in the range of 10^1–10^7 CFU/mL bacteria (E. coli) and fungi (A. niger spore and S. cerevisiae) cells. The fluorescence intensity increased with two microbe cells count's increasing and a linear relationship could be established as the follow ing equations Y = 120.18X−129.89 (R^2 = 0.9951) and Y = 234.07X-254.00 (R^2=0.9960) over the range of 10^2–10^7 CFU/mL.

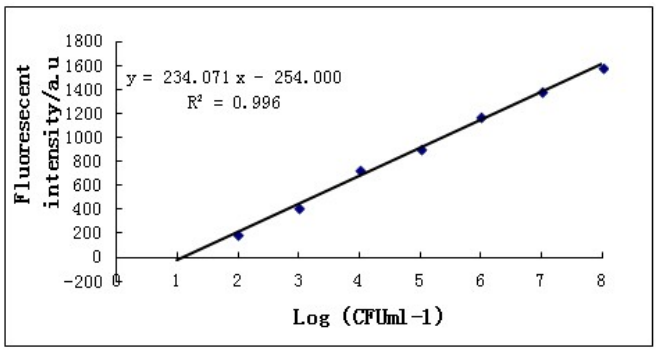

FIG.6 VARIATION IN DIFFERENTIAL FLUORESCENCE INTENSITY WITH TOTAL COUNT OF MIXED A. NIGER SPORE AND S. CEREVISIAE FLUID (REPRESENTED FUNGI)

Determination of Real Samples

The QDs labeled method was used to detect the total bacteria and fungi count of real samples. From Table 1, it could be seen that the values determined using QDs labeled method of the three water samples were similar to the standard plate count method.

TABLE 1 DETERMINATION OF REAL SAMPLES

Water sample	Plate count method (CFU/ml)		QDs coupling method (CFU/ml)	
	bacteria	fungi	bacteria	fungi
Tap water	3.0×10^6	1.0×10^2	2.88×10^6	1.00×10^2
Lake water	4.0×10^6	3.2×10^2	3.86×10^6	3.15×10^2
River water	3.0×10^6	2.0×10^2	2.95×10^6	1.89×10^2

MIC Of Respectivitive Strains

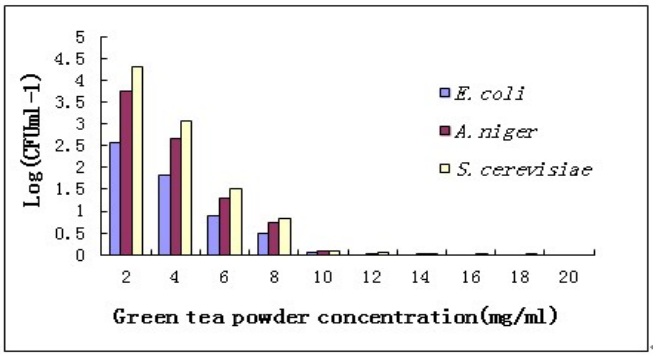

FIG.7 MIC OF E.COLI, A.NIGER SPORE AND S.CEREVISIAE

As shown in Fig.7, MIC of *E.coli, A.niger and S.cerevisiae* was 12, 16, 20mg/ml respectively, it could be seen that

green tea powder had nice antimicrobial activities. Then, it had the best effect on *E.coli*.

Construction of Green Tea Powder Bioreactor and Water Samples Treatment

The schematic diagram of green tea powder bioreactor was shown in Fig.8. Four constituent parts were included, such as the water sample storage, green tea powders column, pump and flow control and treated water samples collector. Meanwhile, in the column, 45cm length green tea powder was installed.

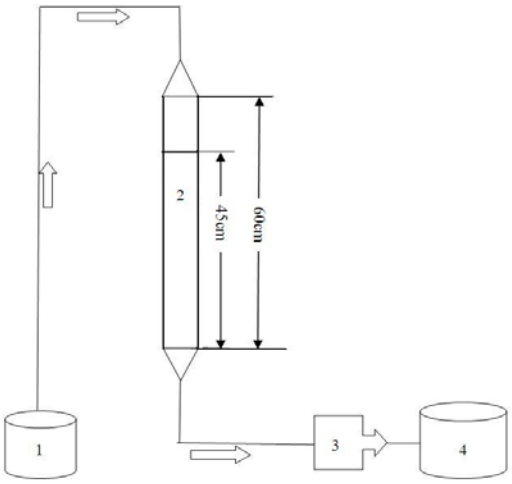

FIG.8 SCHEMATIC DIAGRAM OF GREEN TEA POWDER BIOREACTOR (1-WATER SAMPLE STORAGE, 2-GREEN TEA POWDER COLUMN, 3-PUMP AND FLOW CONTROL, 4-TREATED WATER SAMPLES COLLECTOR)

It could be seen in Fig.9, when the flow rate was no more than 60ml/h, green tea bioreactor could efficiently reduce the bacteria amount of the three samples via adsorbing and destroying bacteria.

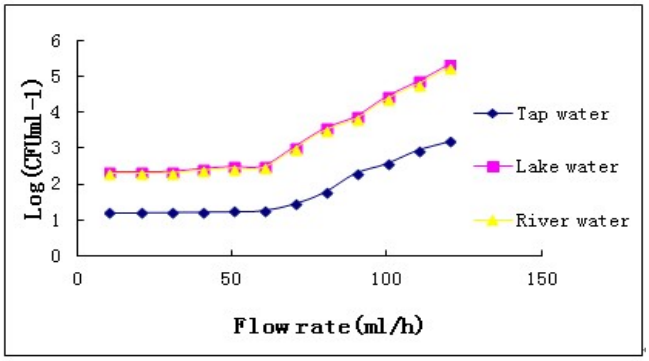

FIG.9 VARIATION OF THE WATER SAMPLES AND BACTERIA COUNT OF TREATED SAMPLE.

Discussion

CdTe QDs and their modified or coated ones were widely used in many different aspects, the most common applications were probe, sign and trace (Dong et al, 2009; Li et al, 2005; Xiao et al, 2010; Zhang et al, 2011), the reason was that CdTe QDs could be connected to many kinds of chemical groups like hydroxyl and carboxyl. As known, microbes' cell wall was rich in these groups that may the precondition for the connection of microbes and QDs. Then, EDC was applied as the coupling agent. We chose E. coli, A. niger spore and S.cerevisiae as the experimental subjects according to their representativeness and universality in their species. To demonstrate that the CdTe QDs were bound to the E. coli, A. niger spore and S.cerevisiae cells, fluorescence microscopy was employed and the fluorescence images were photographed. It could be quite obvious seen that QDs were connected to the three microbe cells well via the comparison of the bright field and fluorescence microscopy.

Many papers had reported that green tea had its effective components such as tea polyphenols (Friedman, 2007; Cho et al, 2010), Epigallocatechin-3-gallate (EGCG) (Sun et al, 2011) had influence on antimicrobial effects. Moreover, green tea has a broad-spectrum antimicrobial activity. For instance, green tea showed excellent antibacterial activity for Listeria monocytogenes (Vodnar, 2012), Pseudomonas aeruginosa (Liu et al, 2012), Helicobacter pylori (Yanagawa et al, 2003), Staphylococcus aureus, Streptococcus pyogenes (Su et al, 2008), Mycobacterium tuberculosis (Guleria et al, 2002), and antifungal activity for Candida species (Hirasawa and Takada, 2004; Park et al, 2006) and Pestalotiopsis theae (Yang and Zhang, 2012). Hence, it was extremely logical to alter green tea as the antimicrobial agent.

In order to identify whether the CdTe QDs bind on the microbe cells, E. coli, A. niger spore and S. cerevisiae were chosen as detection representative target microorganisms because their cell walls had different components. However, they all could be connected to the CdTe QDs as well. It might be explained by the reason that hydroxyl and carboxyl existed in these three kinds of cell walls, with EDC, the covalent bindings occuring.

The sensitivity of QDs coupling fluorescence detection method for directly detecting the total bacteria and fungi count seemed high, and compared with the plates count methods, the data from our results were nearly consistent. Then, contrasting with other popular methods, the sensitivity seemed to be good and effective. Such as, Magliulo et al (2007) have applied multiplexed chemiluminescent immunoassay for the simultaneous detection of Escherichia coli O157:H7,

Yersinia enterocolitica, Salmonella typhimurium, and Listeria monocytogenes, and the limit of the assay quantification were in the order of 10^4 CFU/mL for all bacterial species. Wang et al. (2008) have developed a novel TiO_2 nanowire bundle microelectrode based immunosensor technology for detection of Listeria monocytogenes, and could detect as low as 10^2 cfu/ml of L. monocytogenes in 1 h without significant interference from other foodborne pathogens. Wang et al. (2010) have reported that they have applied FITC-IgG method for L. monocytogenes detection with a detection limit of 50 CFU/mL other strains about 2000 CFU/mL. Yang et al. (2010) have taken a rapid detection of Clostridium perfringens in meat samples with an immunomagnetic separation polymerase chain reaction (IMS–PCR), and with the optimized conditions, the IMS–PCR assay was capable of detecting as few as 10 CFU/g of C. perfringens cells in the meat sample within 10 h. Generally, the QDs method's prominent advantages were that we could spend less time and cost to obtain good determination results.

The new-style green tea bioreactor seemed effective to bacteria. And according to the MIC of green tea, we have provided enough and superfluous green tea podwer in the column. However, as the flow rate further increased to exceed a critical value, the bacteria amount of these samples through the green tea bioreactor also increased, this phenomenon, which was probably due to two possible reasons, firstly, the excessive flow rate could result in the inadequate contact of bacteria with the effective components in green tea. Then, it seem ed that enhancing the flow rate resulted in an increased microorganism growth. On the other hand, the amount of the fungi in these samples through the green tea bioreactor was rather limited and couldn't be accurate ly tested in this method.

In conclusion, in this study, based on the MAA-modified CdTe quantum dots, a simple, rapid, and sensitive fluorescence detection method had been developed for bacteria and fungi counting. The fluorescence method could detect 1×10^2-10^7 CFU/mL bacteria and fungi with a low detection limit and a similar value with the standard plate count method. The fluorescence intensity increased with the increase of the total count of bacteria and fungi, and a linear relationship could be established. Furthermore, the total count of bacteria and fungi in three real samples was determined respectively, the results of determination for the total count of bacteria and fungi were similar with the standard plate count method. It was demonstrated that the application of MAA-modified CdTe quantum dots as fluorescent probes in detecting the total count of bacteria and fungi was feasible.

Green tea powder bioreactor was constructed for treatment of the three real samples, and the results indicated that green tea had nice antimicrobial activities and the bioreactor could control bacteria and fungi amount efficiently and reduce them at flow rate not more than 60ml/h.

Acknowledgments

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