Sensors and Actuators B 216 (2015) 17–23

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Micro corona discharge based cell lysis method suitable for inhibitor resistant bacterial sensing systems

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ARTICLE INFO

Article history:
Received 27 November 2014
Received in revised form 14 February 2015
Accepted 6 April 2015
Available online 20 April 2015

Keywords:
Cell lysis
Corona discharge
Inhibitor resistant
Bacterial detection systems
Ozone

ABSTRACT

We demonstrated a bacterial cell lysis method suitable for inhibitor resistant bacterial detection systems where purification of extracted DNA is not necessary. The presented method potentially improves the field portability of such systems. It allows cell lysis and DNA extraction to be performed without the use of bead mill, sonication, thermal cycling, additional reagents or enzymes. Bacterial cell lysis is achieved in a single step by pumping ozone generated by a micro corona discharge into the bacterial sample. The results with *Pseudomonas putida* as the target bacteria showed that it was capable of achieving 98.5 ± 0.2% lysis (normalized to 1 min of sonication at 10 W) after 10 min of treatment at a flow rate of 38 ml/min and an applied voltage of 2000 V. By increasing the treatment duration, flow rate and applied voltage, the normalized % lysis could be increased. In addition, continuous and pulsed treatments yield similar normalized % cell lysis.

1. Introduction

Inhibitor resistant bacterial detection systems [1–5] herald in the possibility of monitoring water resources in situ for pathogenic contamination. The primary advantage of these bacterial detection systems is that they are able to work with raw environmental samples. Unlike existing bioassays, precipitation and purification during DNA extraction (part of sample preparation) is unnecessary. In other words, DNA extraction for inhibitor resistant bacterial detection systems only requires cell lysis.

Existing DNA extraction methods involve cell lysis, precipitation, and purification through the combination of physical and chemical processes. In particular, cell lysis is often achieved via bead mill [6–10], sonication [11], heating [11,12], and freezing/thawing [11,13], the use of chemical reagents such as EDTA, SDS, and CTAB [11,12,14–16] and enzymes such as lysozyme and proteinase [13,17,18].

As mentioned earlier, both precipitation and purification steps are unnecessary and they result in low DNA recovery yield [19]. Most importantly, the existing cell lysis methods often involve a combination of bead mill, sonication, thermal cycling, additional reagents or enzymes. They can limit the field portability of inhibitor resistant bacterial detection systems for in situ water monitoring. In the case of bead mill, the beads should be removed from the sample prior to DNA hybridization. In the event that the beads are to be re-used, they have to be washed prior to next use. Otherwise they have to be replenished on a regular basis. This will necessitate on-board bead storage and dispensing mechanism. Similarly, the use of additional reagents or enzymes will require storage, preservation, dispensing and replenishment for in situ water monitoring. Thermal cycling within a confinement requires the use of small heating and/or cooling elements. Heating wires often require high electrical currents and high melting point non-electrically conductive materials have to be used to hold them in place. On the other hand, Peltier coolers require relatively large heat sinks to cool effectively. In order to perform fast thermal cycling, both heating and cooling elements have to be used in tandem. More importantly, thermal conduction and insulation at strategic places is critical for efficient heating and cooling. Finally sonication requires the use of piezoelectric materials arranged in specific configurations in order to produce gaseous cavitation for cell lysing. This often requires significant design considerations to achieve meaningful miniaturization outcomes.

In light of the above understanding, we investigated the use of micro corona discharge to generate ozone on demand for bacterial cell lysis in inhibitor resistant bacterial detection systems. As compared to above-mentioned conventional methods, micro

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http://dx.doi.org/10.1016/j.snb.2015.04.030
0925-4005/

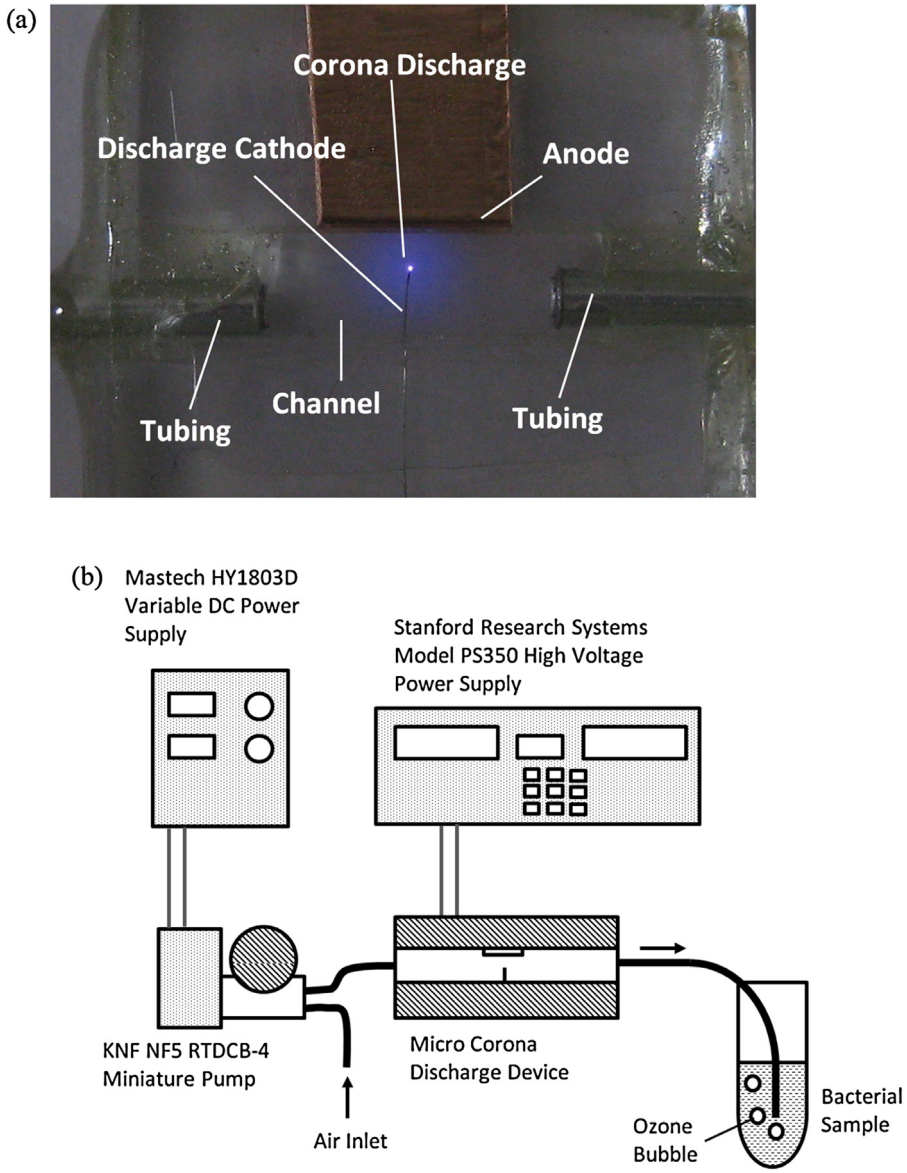

Fig. 1. Micro corona discharge device in operation. (a) Micro corona ionizer in pin-to-plane configuration. (b) Schematic of experimental setup. The system consists of power supplies, miniature pump, micro corona discharge device, and bacterial sample parts.

corona discharge only requires two electrodes housed in line with an air flow that bubbles into the sample. In other words, there is no need for storage, dispensing and replenishment of beads, reagents or enzymes, strategic thermal management or use of piezoelectric materials in specific configurations.

Ozone is a well-known bactericide, which caused the destruction of the cell wall and membrane [20–25]. Scott and Lesher postulated that the primary attack of ozone on the cell wall or membrane, probably by reaction with the double bonds of lipids [21]. Bringman reported that ozone acted as a general protoplasmic oxidant [22,23]. Curtiellas et al. have shown the large scale feasibility of bacterial cell lysis by bubbling ozone into the sample [26]. This results in a change in cell permeability which eventually leads to cell lysis [25]. Cell surface damage by ozone is often evidenced by protein release, lipid peroxidation and change in cell permeability [21]. By employing a micro corona discharge device, we harnessed the same cell lysis principle and scaled it down such that it is suitable for field portable bacterial detection systems. In this paper, we demonstrated the efficacy of ozone generated by a micro corona discharge device for bacterial cell lysis (*Pseudomonas putida* as the target bacteria). Bacterial cell lysis was verified both visually by field emission-scanning electron microscopy as well as via experiment. Operation parameters such as flow rate, applied voltage and treatment time were characterized to identify the operation envelope. During the autonomous operation of field portable bacterial monitoring systems, intermittent power disruption is a possibility. In this case continuous treatment of bacterial samples might not be possible. Therefore we also established the equivalence between pulse and continuous treatments.

2. Materials and methods

The micro corona discharge device composed of a discharge cathode and an anode arranged in a pin-to-plane configuration as shown in Fig. 1a. A 50 μm diameter stainless steel wire was employed as the discharge cathode and a 50 μm thick copper foil was employed as the anode. Both discharge cathode and anode were housed within a cut glass channel and sealed with epoxy. Aluminum tubings were attached to both ends of the channel to facilitate connection with air tubings (HelixMark 60-825-37,