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Detection of Cryptosporidium Oocysts in Micro X-ray Computed Tomography Scans

Fei Duan[1]* and Tong Liu[2]

[1]Division of Thermal and Fluids Engineering, School of Mechanical and Aerospace Engineering, Nanyang Technological University, 639 798 Singapore.
[2]Precision Measurements Group, Singapore Institute of Manufacturing Technology, 638 075 Singapore.

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The water containing viable Cryptosporidium oocysts was scanned before or after the microwave heating under a micro X-ray computed tomography system. Cryptosporidium oocysts clusters, found in the specimens without the heating, vanished after 30-second heating when the water temperature approached 100 degree C. After the aqueous specimens were dried, the image analysis indicated that the dried remainder in the specimen without the heating was darker and in rectangular shapes, but whiter and irregular in the specimen after 30-second heating. The preliminary results suggest the low-energy X-ray source should be applied for the aqueous specimens.

Key words: Microwave heating; micro X-ray computed tomography scan; Cryptosporidium oocysts; water.

One of important waterborne microbes to jeopardize the public health is Cryptosporidium (Ware *et al.* 2003), a protozoan organism which can cause the parasitic infection, cryptosporidiosis. Ingestion of water contaminated with viable Cryptosporidium oocysts is the major mode of transmission. In the past decades Cryptosporidium oocysts have been regarded as common contaminants in the drinking water supply. Half of millions of individuals have been affected with the outbreaks of cryptosporidiosis (Smith and Rose 1998). Thus, accurate and fast detection of these pathogens is critical to monitor water quality. Various staining techniques have been developed to assist microbiologists in the identification. One widely used method is the fluorescence assay, which employs an antibody tagged with a fluorescent dye to locate the viable oocysts under fluorescence microscopy (Ware *et al.* 2003; Hijjawi *et al.* 2004). However, the detecting process requires a long-time preparation of the specimens. The traditional approved standard methods for waterborne microbial detection are normally time-consuming. Two

* To whom all correspondence should be addressed.
Tel: +65-67905510; Fax: +65-67924062
E-mail: feiduan@ntu.edu.sg

dimensional scans under a micro X-ray computed tomography (CT) system, a nondestructive materials testing method, is proposed to apply in detecting viable Cryptosporidium oocysts in the density shadow of X-ray beam. Although commonly used in medical science, X-ray CT scanner has been used in the biological studies. Parkinson *et al.* (2008) developed soft X-ray tomography to image the cells in biological and biomedical researches. In the paper, the preliminary experimental X-ray tomography detection of viable Cryptosporidium oocysts in pure water is introduced. Microwave heating was applied to treat the aqueous specimens to visualize the X-ray detection capacity on the viability of Cryptosporidium oocysts.

MATERIAL AND METHODS

The original viable Cryptosporidium oocysts are from infected calves (Cryptosporidium parvum oocysts live, 5 million, Iowa isolate). The viable oocysts were stored in the storage solution at 4 °C until they were mixed with pure water for the experiments. The loss of viability of Cryptosporidium oocysts is assumed to be negligible in preparing specimens except microwave heating and drying.

The specimens were well mixed of 1 ml original viable Cryptosporidium oocyst solution and 4 ml nano-filtered, deioned water with the help of a shaker to eliminate the effects of other possible particles firstly. Parts of the diluted Cryptosporidium oocysts specimens were then heated in a microwave oven (Sanyo, 1100 kW) once they were promptly prepared. The heated aqueous specimens at 17 s and 30 s were compared with those without the microwave heating treatment. Afterwards, 0.2 ml of aqueous specimens was immediately transported into a BD utensil for two dimensional scans under a micro X-ray CT system, FeinFocus Fox 160.25 at Singapore Institute of Manufacturing Technology of A*Star, as sketched in Fig. 1. An open tube X-ray source, of which the voltage and current of the X-ray source are adjustable, can release X-ray radiation beams to pass through the specimen from the top. The density shadow is captured by a digital direct detector below. The two dimensional images were recorded in a computer, which is also used to drive a mechanical control system to visualize different portions at the specimen. The scans could be finished in a second level. After the scans, the specimens were dried under a fan at room temperature in an isolated clean container. Finally, the dried remainders were rescanned in the micro X-ray CT system to confirm the existence of Cryptosporidium oocysts under different microwave heating histories. The specimens with different microwave heating treatments were also examined under an optical microscope (Zeiss).

RESULTS AND DISCUSSION

Microwave heating was selected in the study because it is an efficient method to heat the water up to a certain temperature rapidly. The heating treatment in a Sanyo microwave oven demonstrates that the temperature in the aqueous specimens (5 ml) could be greater than 80 °C after 15-second heating in Fig. 2. If the heating time was at 30 s, the water temperature approached 100 °C. The result is consistent with the investigation of Ortaga and Liao (2006). They found that the viable Cryptosporidium oocysts could be completely inactivated within 20-second microwave heating when the liquid temperature of 80 °C or higher was reached. The optical microscopic images were applied to differentiate Cryptosporidium oocysts in the pure water. Before the microwave heating, both single Cryptosporidium oocysts and Cryptosporidium oocysts clusters were observed, as shown in Fig. 2(a), however, after 30-second microwave heating, there were still some measureable objects found in the image of Fig. 2(b) although the viable Cryptosporidium oocysts were believed to be inactivated (Ortaga and Liao 2006). It suggests that the normal optical microscopy is insufficient to take apart viable and inviable Cryptosporidium oocysts. As we known, optical microscopes differentiate particles based on the various reflection of light, but micro X-ray CT scanners differentiate particles based on the density variation.

Two dimensional micro X-ray CT scans were conducted for the series of aqueous specimens. As seen in Fig. 3(a), some irregular dark spots, marked in circles, were found in the

liquid of the specimens, which were not heated in the microwave oven. Note that these specimens were a mixture of nano-filtered, deioned, pure water and viable Cryptosporidium oocysts before the heating treatments. The dark spots with a size at around 10-50 mm in Fig. 3(a) should be related to the viable Cryptosporidium oocysts. Since single Cryptosporidium oocysts are about 3-6 µm, the larger size spots in the density shadows were supposed to be the clusters of viable Cryptosporidium oocysts, which were also seen in the optical microscopic image in Fig 2(a). After heated for a certain time in the microwave oven (17 s or 30 s), the specimens were also scanned in the micro X-ray CT system. The images, shown in Figs. 3(b) after 17-second heating and 3(c) after 30-second heating, were compared with the one without microwave heating. As seen there, the visible Cryptosporidium oocyst clusters became less in number and weaker in color in the aqueous specimen after 17-second microwave heating. No Cryptosporidium oocyst clusters were detected after 30-second heating. Although the single Cryptosporidium oocysts in the aqueous specimens cannot be detected in the present micro X-ray CT system, it cannot deny their existence. The aqueous specimens were dried up to eliminate the water effects in the detection of the Cryptosporidium oocysts. The same amount of aqueous specimens after the heating treatment at 0 s, 17 s, and 30 s were dried under a fan at room temperature in a clean surrounding. Then, the dried specimens were rescanned in two dimensions under the micro X-ray CT system. Interestedly, the reminder of the specimen without the microwave heating, which had the viable Cryptosporidium oocysts originally, formed a series of rectangles on a flat plate in the two dimensional view as shown in Fig. 3(d). A partition space was found between each pair of adjacent layers. The area was the largest among the three dried specimens, and the color of the sediment was the darkest. After 17-second microwave heating, the reminder was in a sector shape with the partition lines in Fig. 3(e). The area was smaller than the specimen without microwave heating. There were not any partition lines after 30-second microwave heating. As shown in Fig. 3(f), the shape was irregular, the area was the smallest, and the color was the

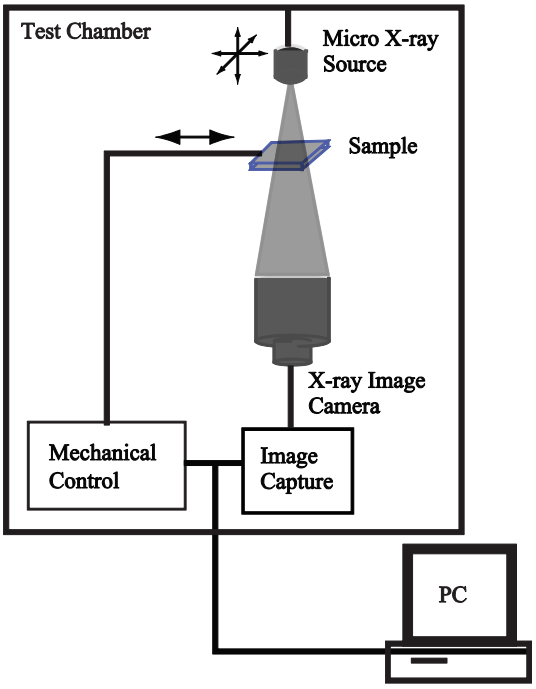

Fig. 1. The sketch of the micro X-ray CT system. The specimens were scanned in two dimensions

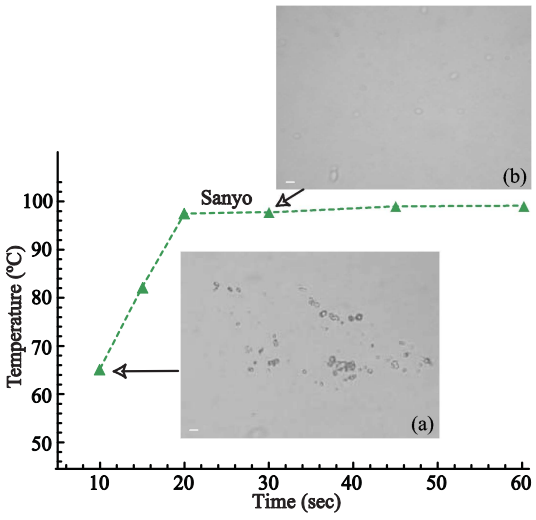

Fig. 2. The liquid temperature as a function of the microwave heating time. The inserted optical microscopic image (a) is for the specimens before the microwave heating, and the image (b) is for the specimen after 30-second heating. The scale is in 10 µm.

weakest. It is indicated that the viable Cryptosporidium oocysts can react when the aqueous specimens were dried, and became less and less with an increase of the microwave heating time. After 30-second heating, the temperature of the aqueous specimen reached 100 °C, at which the viable oocysts were inactive (Ortaga and Liao 2006). Thus, the dried sample showed the weakest color in an irregular configuration. That the viable Cryptosporidium oocyst clusters were not detectable in the water in Fig.3(c) might be resulted from that the destroyed Cryptosporidium oocysts was empty or became fragment when the water temperature reached 100 °C, and the empty or dead Cryptosporidium oocysts have lower density, close to water (Ware *et al.* 2003; Young and Komisar 2005). But in the specimens without microwave heating, the viable Cryptosporidium oocyst clusters could be detected because the viable clusters have a higher density. Consequentially, the dried sample was much darker. The regular sediment also gives us some hint on the viability of Cryptosporidium oocysts in that specimen.

It is known that the density of water is about 997 kg/m^3 at room temperature and that of viable Cryptosporidium oocysts is around 1077 kg/m^3 (LeChevallier *et al.* 1995; Young and Komisar 2005). The density difference between viable Cryptosporidium oocysts and water is above 8%, thus, it provides a potential method to detect

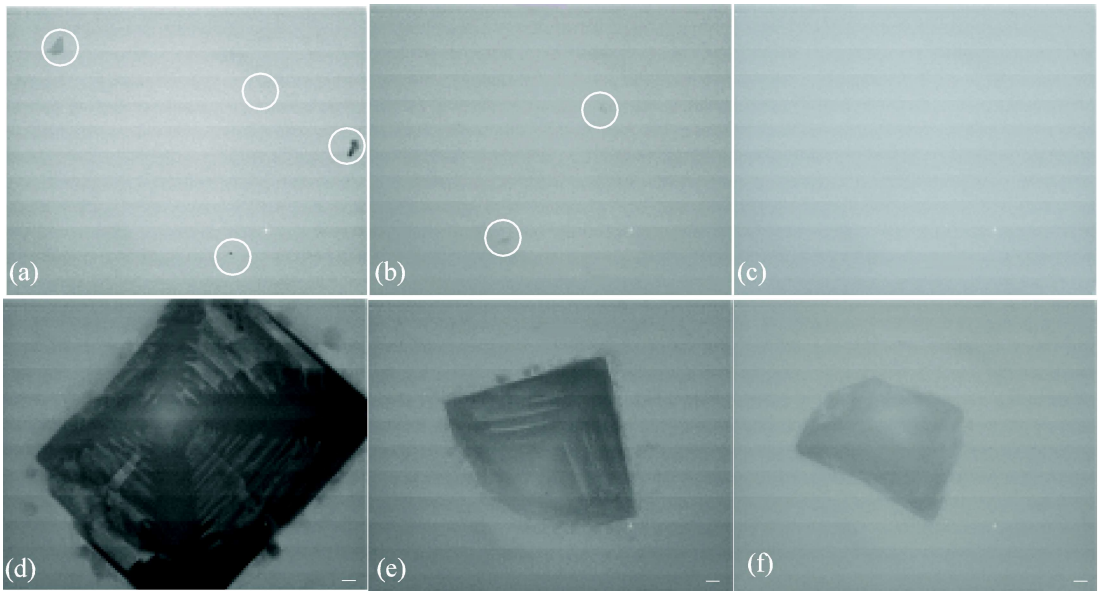

Fig. 3. The two dimensional micro X-ray CT scanning images of the specimens, (a) no microwave heating of the aqueous specimens, (b) 17-second microwave heating of the aqueous specimens, (c) 30-second microwave heating of the aqueous specimens, (d) no microwave heating of the dried specimens, (e) 17-second microwave heating of the dried specimens, and (f) 30-second microwave heating of the dried specimens. The scale is in 50 μm

viable Cryptosporidium oocysts from water in the two dimensional X-ray CT scans. However, the reason that no single viable Cryptosporidium oocysts were found in Fig. 3(a) is probably from the limitation of the micro X-ray CT system used in the preliminary experimental studies. The CT system is designed for electronic applications. The linear response of the digital direct detector to X-ray source is in the range from 45 to 160 kV. When the X-ray tube voltage is below 45 kV, the detector becomes insensitive. However, the voltage at these two-dimensional X-ray scans had to be set at 45 kV although we knew that 45 kV X-ray energy would be too high to generate good enough contrast between viable Cryptosporidium oocysts and water in the aqueous specimens. As a result, although some Cryptosporidium oocyst clusters were detected in the scans, single

Cryptosporidium oocysts were difficult to be observed as those shown in the inserted Fig. 2(a). However, the problem might be solved by using a nano-X-ray source (5kV to 20kV), which could supply a two dimensional lateral resolution as small as 200 nm for differentiating single Cryptosporidium oocysts with a size of 3-6 μm from water. Correspondingly, a direct digital detector for this range of X-ray energy would be selected as well.

CONCLUSIONS

To detect viable Cryptosporidium oocysts in pure water, the specimens with the different microwave heating treatments were scanned in two dimensions under the micro X-ray CT system. The image shows that the viable Cryptosporidium oocyst clusters vanished after they were heated for 30 s while the viable Cryptosporidium oocyst clusters were visible in the aqueous specimens without heating (Fig. 3). After the specimens were dried, the remainder in the specimen without the microwave heating was the darkest and in rectangular shapes partitioned by a series of space, but the weakest and in irregular shapes after 30-second heating (Fig. 3). The present study made a preparation for the X-ray detection to be applied in drinking water since the denser inorganic particle could generate larger contrast in the X-ray shadow from water and Cryptosporidium oocysts once the X-ray source and detector are optimized in future. The further investigation would be proofed by the traditional viability assessment of Cryptosporidium oocysts.

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