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ANTIBACTERIAL EFFICIENCY OF BORON DOPED DLC COATINGS

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ABSTRACT

B (boron) doped DLC coatings with different B contents (4.9 at.%, 7.6 at.% and 9.5 at.%) were prepared on stainless steel substrates by plasma-enhanced chemical vapour deposition (PECVD) technique. The experimental results showed that the surface energy of the B-DLC coatings decreased with increasing B content in the coatings. The incorporation of Boron into the DLC coatings reduced significantly bacterial attachment and increased bacterial removal. The numbers of adhered bacteria decreased with boron content increasing or with the total surface energy of the B-DLC coatings decreasing. The 9.5% B-DLC performed best, which reduced total bacterial adhesion by up to 70%, compared with pure DLC coating. In order to assess the adhesion strength of the attached bacteria, some bacteria on the coatings were removed through a dipping process. The removal percentage of the bacteria increased with boron content increasing or with the total surface energy of the B-DLC coatings decreasing. The removal percentage of the bacteria on the 9.5% B-DLC coatings increased by 25%, compared with pure DLC coatings.

INTRODUCTION

Micro-biofouling has been recognized as a widespread problem in the design and operation of processing equipment, especially in heat exchangers. An effective and desired approach to reduce biofouling is to alter the surface properties of heat exchangers by surface coating techniques. Many attempts have also been made to reduce fouling by coating surfaces with polymers (e.g. silicone, PTFE) due to their non-stick properties. However, the poor thermal conductivity, poor abrasion resistance and poor adhesion to metal substrate of these polymer coatings currently inhibit their commercial use.

Diamond-like carbon (DLC) coatings have attracted great interest because of their properties such as good thermal conductivity similar to metals, low friction, extremely smooth surface, hardness, wear resistance and corrosion resistance (Grill, 1993), which make them suitable for heat exchanger applications. The thermal conductivity of diamond-like carbon coatings is about 3.5 W/m.K (Shamsa et al, 2006). The thickness of the DLC or B-DLC coatings is only about 2 micrometers, so any additional thermal resistance is negligible.

DLC itself does not have particularly good non-stick properties. The amorphous nature of DLC opens the possibility of introducing additional elements, such as Si, F, N, O and their combinations, into the coating whilst still maintaining the amorphous phase of the coating (Hauert 2003). Recently the incorporation of selective elements into DLC has been shown to be an effective method to alter the surface energy of the DLC coatings and to enhance the antibiofouling and other properties of DLC coatings. Zhao et al. (2007) showed that the silicon-doped DLC coatings reduced bacterial attachment significantly compared with pure DLC coatings. Numerous studies demonstrated that the heat transfer surfaces coated with fluorine-doped DLC coatings reduced scale adhesion and subsequent scale formation, compared with DLC coatings, untreated titanium and stainless steel surfaces (Müller-Steinhagen and Zhao, 2000; Bornhorst et al, 1999; Santos et al. 2004; Zhao and Wang 2005). It was also demonstrated that the antibacterial performance of the pure DLC coatings was improved significantly by the incorporation of fluorine (Ishihara et al. 2006) or titanium (Zhao et al, 2013) with *Escherichia coli*. However, there are no studies that explore bacterial adhesion and removal with boron doped DLC coatings. In this study, boron doped DLC coatings were prepared and evaluated for bacterial adhesion and removal.

MATERIALS AND METHODS

Boron-doped DLC coatings

DLC coating manufacture is well established. For doped DLC coatings, the selection of the doped elements for anti-fouling application were mainly based on previous experience on producing non-stick doped DLC coatings and on the ability of manufacturers to produce the coatings with consistent quality after a period of experimentation and development using a PECVD process, which is feasible for application to heat exchangers. In the present study, B-doped DLC coatings with different B contents (4.9 at.%, 7.6 at.% and 9.5 at.%) were prepared on stainless steel substrates (25×25×1mm) by a plasma-enhanced chemical vapour deposition (PECVD) in order to investigate the effect of B contents on the adhesion and removal of bacteria.

Surface Energy of B-DLC Coatings

The surface energy of a solid surface gives a direct measure of intermolecular or interfacial attractive forces. van Oss et al (1988, 1994) proposed to divide the total surface energy of a material (γ_i^{TOT}) into 3 independent components, Lifshitz-van der Waals apolar (γ_i^{LW}), the electron acceptor (γ_i^{+}) and the electron donor (γ_i^{-}):

$$\gamma_i^{TOT} = \gamma_i^{LW} + 2\sqrt{\gamma_i^{+}\gamma_i^{-}} \qquad (1)$$

In this study the contact angles on the B-DLC coatings were obtained using a sessile drop method with a Dataphysics OCA-20 contact angle analyser at the Biological and Nanomaterials Lab, University of Dundee.

Assays of Bacterial Adhesion and Removal

In this study, the suspension of *Pseudomonas aeruginosa* with a 10^6 CFU/ml concentration was prepared and used for bacterial adhesion assays. Five replicate samples of each coating were immersed vertically in a glass tank containing 500 ml of a bacterial suspension and were incubated on a shaker at 20 rpm for 1 hour at 37˚C. The total number of bacteria on the samples was counted by a BX41 Olympus Fluorescence Microscope with QICAM High-Performance Digital CCD Camera after the samples were exposed to air for 15 minutes at 25˚C. The LIVE/DEAD *BacLight* bacterial viability kit was used for the enumeration of bacteria on the coatings. The kit consists of two nucleic acid stains: SYTO 9, which penetrates most membranes freely, and propidium iodide, which is highly charged and normally does not permeate cells but does penetrate damaged membrane. Simultaneous application of both dyes therefore results in green fluorescence of viable cell with an intact membrane, whereas dead cells, because of a compromised membrane, show intense red fluorescence. The number of bacteria is counted automatically using image analysis by *Image Pro Plus*. The green colour shows alive bacteria while the red colour shows dead bacteria. 8 fields (approximate 48000μm^2) were used for microscope image analysis.

In order to investigate bacterial adhesion strength, a home-made dipping device was designed by Zhao *et al.* (2008). It is designed to remove 10% ~ 90% bacteria from surfaces through moving the sample up and down into the water at constant speed (0.03 m/s) controlled by a motor. This process is identical to water flowing over the surface of the sample and the whole area of samples is flushed with water at constant shear stress. Therefore, the bacteria adhered weakly to the surfaces can be removed into the water tank. Finally, bacterial adhesion strength and removal percentage can be determined (Zhao et al 2008; Liu 2011).

RESULTS

Contact angle and surface energy

Table 1 shows the surface energy components of the coatings, which were calculated by van Oss acid-base approach with the contact angle data.

Table 1. Surface energy components of B-DLC coatings

Coatings	Surface Energy Components [mJ/m^2]			
	γ^{LW}	γ^{+}	γ^{-}	γ^{TOT}
DLC	43.56	0.35	4.47	46.06
4.9%B-DLC	39.36	0.40	7.55	42.84
7.6%B-DLC	33.94	0.00	2.03	33.94
9.5%B-DLC	28.99	0.00	4.73	28.99

Bacterial Adhesion

The percentage of live cells on the suspension prior to adhesion was over 99%. Figures 1 and 2 show the attachment of *P. aeruginosa* cells on the DLC coating and B-DLC coatings for contact time 1 hour. After the coatings with bacteria were exposed to air for 15 minutes, some bacteria on the coatings were dead. It was found that the difference between the number of live bacteria and the number of dead bacteria on DLC coating at 15 minutes was statistically important. However the dead number increased with increasing time. All the B-DLC coatings performed much better than the pure DLC coating against bacterial attachment. The number of adhered bacteria (live, dead and total bacteria) decreased linearly with B content increasing (Figure 2). The 9.5% B-DLC performed best, which reduced total bacterial adhesion by 70%, compared with the pure DLC coating.

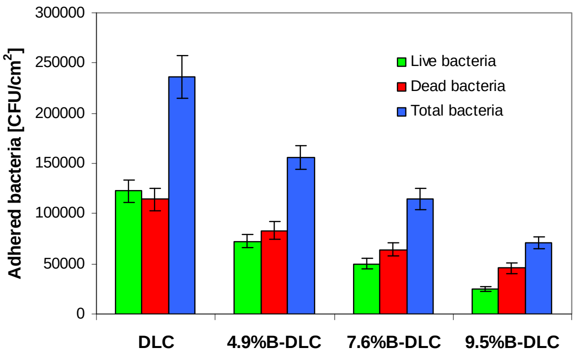

Figure 1 Attachment of *P. aeruginosa* cells on coatings

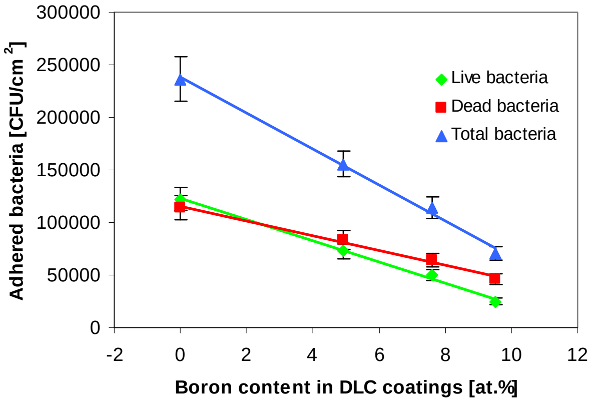

Figure 2 Effect of Boron content on the attachment of *P. aeruginosa* cells on B-DLC coatings

Bacterial Removal

In order to assess the adhesion strength of the attached bacteria, each sample with bacteria was dipped 20 times vertically in a glass vessel containing 130 ml sterile distilled water at 37 °C at constant speed (0.03 m/s) controlled by a motor with a constant shear stress of 0.014 N m^{-2}. During the dipping process, some bacteria were removed from the coatings. Figure 3 shows the numbers of remaining bacteria on the coatings. The numbers of remaining bacteria (live, dead and total bacteria) decreased with boron content increasing. The remaining bacteria (live, dead and total bacteria) on the 9.5% B-DLC coatings were reduced by 87%, 77% and 83% respectively, compared with pure DLC coating. Figure 4 shows that the numbers of remaining bacteria (live, dead and total bacteria) decreased linearly with B content increasing.

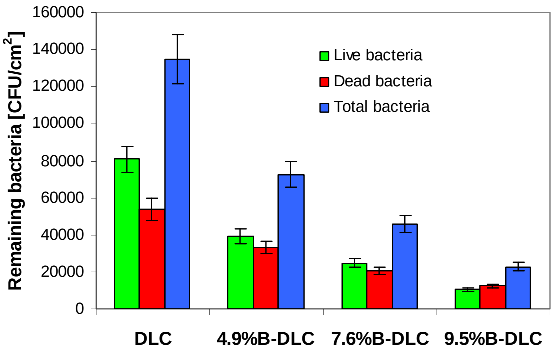

Figure 3 Remaining *P. aeruginosa* cells on the coatings

Figure 5 shows the removal percentage of *P. aeruginosa* cells from the B-DLC coatings after dipping process. The removal percentage of live, dead and total bacteria increased with boron content in the DLC coating increasing. The removal percentage of live, dead and total bacteria on the 9.5% B-DLC coatings was 58%, 73% and 68% respectively; while the removal percentage on the pure DLC coatings was only 34%, 53% and 43%. The removal percentage of the total bacteria on the 9.5% B-DLC coatings increased by 25%, compared with pure DLC coatings. Figure 5 also indicates that the removal percentage of dead bacteria was higher than that of live bacteria. This means the dead bacteria were more easily removed than the live bacteria.

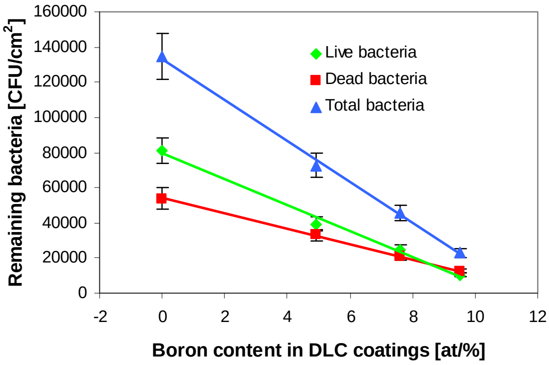

Figure 4 Effect of B content in DLC coatings on the remaining *P. aeruginosa* cells

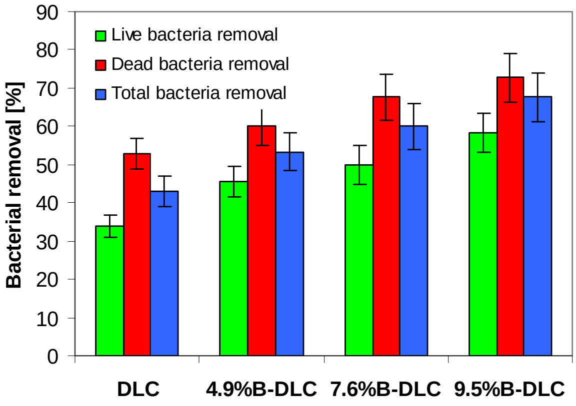

Figure 5 Removal percentage of *P. aeruginosa* cells from B-DLC coatings after dipping process (Contact time: 1 h)

DISCUSSION

The improved anti-bacterial properties of B-DLC coatings with B content increasing could be due to total surface energy decreasing. Table 1 clearly indicates that the total surface energy of B-DLC coatings decreases with B content increasing.

Figure 6 shows that the live, dead and total *P. aeruginosa* cells on the B-DLC coatings decreased with the total surface energy of the B-DLC coatings decreasing. The results were consistent with previous results on the effect of total surface energy on bacterial adhesion. If the total surface energy approaches to 25 mJ/m^2, bacterial adhesion is minimal (Liu & Zhao, 2011).

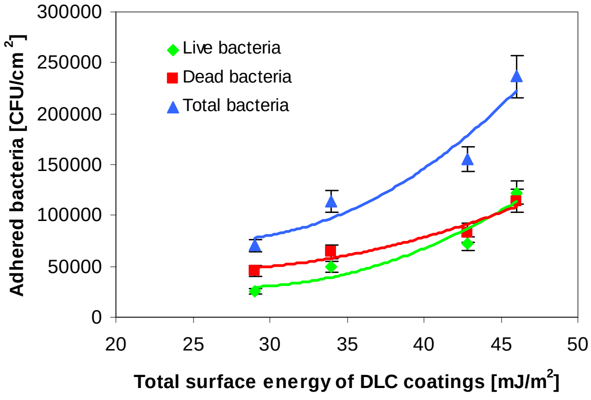

Figure 6 Effect of surface energy of B-DLC coatings on the attachment of live, dead and total *P. aeruginosa* cells (Contact time: 1 h)

Figure 7 shows that the remaining *P. aeruginosa* cells (live, dead and total bacteria) on the B-DLC coatings decreased with the total surface energy of the B-DLC coatings decreasing. Again if the surface energy approaches 25mJ/m^2, the number of the remaining bacteria is minimal (Liu & Zhao, 2011).

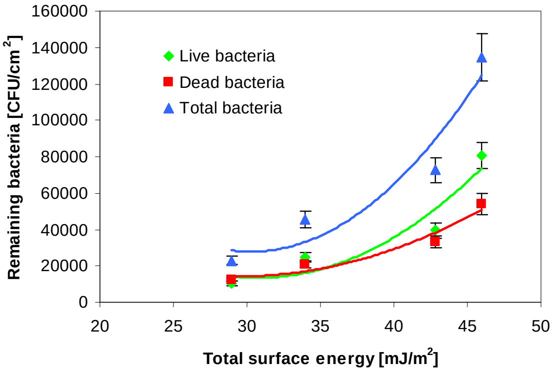

Figure 7 Effect of surface energy of B-DLC coatings on the remaining *P. aeruginosa* cells (Contact time: 1 h)

CONCLUSIONS

- A series of new B-doped DLC coatings with different contents of Boron (4.9, 7.6 and 9.5%) were designed and produced on stainless steel substrates. The experimental results showed that the total surface energies of B-DLC coatings decreased with B content increasing.
- It was observed that after the coatings with bacteria were exposed to air for 15 minutes, some bacteria on the coatings were dead.
- The numbers of adhered bacteria (live, dead and total *E. coli* cells) decreased with B content increasing or with total surface energy decreasing. The 9.5% B-DLC performed best, which reduced total bacterial adhesion by 70%, compared with pure DLC coating.
- The removal percentage of the bacteria (live, dead and total bacteria) increased with B content increasing or with total surface energy decreasing. The remaining bacteria (total bacteria) on the 9.5% B-DLC coatings were reduced by 83%, compared with pure DLC coating. The removal percentage of the total bacteria on the 9.5% B-DLC coatings increased by 25%, compared with pure DLC coatings.

NOMENCLATURE

γ^{LW}	Lifshitz-van der Waals component of surface energy, mJ/m^2.
γ^{TOT}	Total surface energy, mJ/m^2.
γ^{-}	Electron-donating parameter of the acid-base component, mJ/m^2.
γ^{+}	Electron-accepting parameter of the acid-base component, mJ/m^2.

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