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ANTIMICROBIAL EFFICIENCY OF METAL-POLYMER NANOCOMPOSITE COATINGS

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ABSTRACT

The anti-microbial efficiency of a range of biocide polymers was evaluated. It was found that SM14 and SM20 performed best against bacterial attachment and adhesion. The two polymers SM14 and SM20 were incorporated into Ni-P and Ni-P-PTFE coatings using an electroless plating technique and four new coatings were developed: Ni-P-SM14, Ni-P-PTFE-SM14, Ni-P-SM20 and Ni-P-PTFE-SM20. The research results indicated that the contents of SM14, SM20 and PTFE had significant influence on antimicrobial efficiency. The coatings with both biocide polymers and PTFE (Ni-P-PTFE-SM14 and Ni-P-PTFE-SM20) performed best against bacterial adhesion, which reduced the attachment of *Escherichia coli* and *Staphylococcus aureus* by 92% and 86%, respectively, as compared with stainless steel. It was also found that for the Ni-P-PTFE coatings the number of adhered bacteria had strong correlation with CQ ratio, which is defined as the ratio of Lifshitz-van der Waals apolar component γ^{LW} to electron donor component γ^{-} of substrates. The research results demonstrated that biocide polymer and the CQ ratio are key factors for controlling bacterial attachment, which gives us a clear direction for the design of anti-biofouling coatings through surface modification.

INTRODUCTION

There is a common need for preventing and eradicating microbial contamination across the supply and production chain in the food industry, especially in the food processing industries. Biofouling does not only present a considerable hygiene risk in the food industry, but also causes economic losses. Any method of preventing biofouling or lengthening processing time will give substantial cost benefits. Since bacterial adhesion is a prerequisite condition for biofilm formation, prevention of bacterial adhesion and colonization on the processing equipment will have a major impact in preventing biofouling.

Electroless nickel-phosphorus coatings have been widely applied in many industries, such as food processing and chemical engineering etc. due to their good corrosion and wear resistance (Zhao *et al*, 2002). Polytetrafluoroethylene (PTFE) is chemically very inert and has a relatively high melting point (325 °C). PTFE also has non-stick property due to its low surface energy. Its coefficient of friction (0.05) is lower than that of almost any other polymer (Zhao *et al*, 2002). The incorporation of PTFE nanoparticles into the Ni–P matrix can take advantage of the different properties of Ni–P alloy and PTFE. The resulting properties of electroless Ni–P–PTFE coatings, such as non-stick, anti-adhesive, lower friction, good wear and good corrosion resistance, have been used extensively in many industries (Ger *et al*, 2002; Zhao *et al*, 2002). Because electroless Ni-P-PTFE coatings are metal-based, their thermal conductivity, anti-abrasive property and mechanical strength are superior to standard PTFE coatings (Zhao *et al*, 2004, 2005, 2006).

Over the past two decades, bacterial adhesion to surfaces with different surface energies has been investigated intensively with the frequent conclusion that bacterial adhesion is less to low energy surfaces and easier to clean because of weaker binding at the interface . In order to investigate the influence of surface energy components of substrates on bacterial adhesion, the adhesion of both freshwater bacterium and marine bacteria to the Ni-P-PTFE coatings with different surface energies were performed. The anti-microbial efficiency of a range of biocide polymers which were developed by Graz University of Technology was evaluated with *Escherichia coli* and *Staphylococcus aureus*. Then the selected polymers were incorporated into Ni-P-PTFE coatings using an electroless plating technique. The antimicrobial efficiency of the coatings with biocide polymers was evaluated with *Escherichia coli* and *Staphylococcus aureus.*

MATERIALS AND METHOD

Ni–P–PTFE–biocide polymer composite coatings

The stainless steel samples were first cleaned with alkaline solution at 60–80°C for 10–20 minutes and then rinsed with water. The composition of the alkaline solution included 25 g/l NaOH; 25 g/l Na_2CO_3; 30 g/l Na_3PO_4 and 8 g/l Na_2SiO_3. The samples were dipped into a dilute HCl solution (1 M) for 30 s and then rinsed with cold water and deionized water, respectively. A 60% PTFE emulsion from Aldrich with a particle size in the range 0.05–0.5 µm was diluted with deionized water and stirred with a magnetic stirrer for 1 hour. Then the solution was filtered with a filter of pore size 0.2 µm before use. Then Ni–P–PTFE composite coating was prepared using an electroless plating technique. The Ni–P–PTFE–biocide polymer composite coatings were also prepared using this technique, by adding biocide polymers which were developed by Graz University of Technology (Kreutzwiesner and Noormofidi *et al*, 2010).

Contact angle and surface free energy

The contact angle of the coatings was measured by using sessile drop method with a Dataphysics OCA-20 contact angle analyzer. This instrument consists of a CCD video camera with a resolution of 768×576 pixel and up to 50 images per second, multiple dosing/micro-syringe units and a temperature controlled environmental chamber. The drop image was processed by an image analysis system, which calculated both the left- and right contact angles from the shape of the drop with an accuracy of ± 0.1 °. Three test liquids were used as a probe for surface free energy calculations: distilled water, diiodomethane (Sigma) and ethylene glycol (Sigma). All measurements were made at 25 °C. Surface free energies of the samples and their dispersive and polar components were calculated using van Oss acid–base (AB) approach (van Oss *et al*, 1988).

Bacterial adhesion assay

In this investigation *Escherichia coli* and *Staphylococcus aureus* from Institute of Infection and Immunity, Nottingham University, UK was used for bacterial adhesion assays to Ni-P-PTFE-biocide polymers. Samples were put into a beaker containing 100ml bacterial suspension with 1×10^7 CFU/ml concentration and then the beaker was put in a shaker incubator (Stuart Scientific, UK) at 37°C for 1h under a gentle stirring at 20rpm. In order to observe the number of cells microscopically, the bacteria on the samples was stained using LIVE/DEAD BacLight Kit L 13152 for 15 minutes and then observed under a fluorescence microscope (OLYMPUS BX 41, Japan) and counted using Image Pro Plus software (Media Cybernetics, USA).

In order to investigate the effect of surface energy on bacterial adhesion, another 3 types of bacteria, *P. fluorescens, Cobetia* and *Vibrio* were used for bacterial adhesion assays to the Ni-P-PTFE coatings. After the frozen bacteria stock had been defrosted, they were cultured in tryptone soya ager (TSA) plates in an incubator overnight at 28°C. A single colony was taken and put into 10ml medium to grow overnight at 28°C. The medium for *P. fluorescens* was tryptone soya broth (TSB) and the medium for *Cobetia* and *Vibrio* was sea salt peptone (SSP) containing peptone 18 g/l and sea salt 40g/l. Then 500µl of each bacterial suspension was transferred into 100ml corresponding medium in a conical flask and cultured in a shaker-incubator at 150rpm at 28°C until the bacteria grew up to mid-exponential phase. Then the bacterial suspension with a 10^7 CFU/ml concentration for each type of bacteria was prepared. For freshwater bacterium, the phosphate buffered saline (PBS) was used and for marine bacteria SSP containing nutrient was used. For bacterial adhesion assays, each sample was immersed in a tank containing 80ml bacterial suspension at 28°C for 6hr, 12hr, and 24hr, respectively. Then each sample was taken out for viable plating count to determine the number of adhered bacteria on the samples.

RESULTS

Anti-microbial efficiency of biocide polymers

The anti-microbial efficiency of 13 different types of biocide polymers was evaluated with *Escherichia coli* and *Staphylococcus aureus*. Figure 1 shows that all the polymers have strong antimicrobial properties with respect to the bacterium *Escherichia coli*, compared to the polymer without additives (355.09.012). The polymers 355.09.004; 355.09.006; SK13; SM14 and SM20 perform better than others against bacterial attachment. Figure 2 shows that the biocide polymers have strong antimicrobial properties with respect to the bacterium *Staphylococcus aureus*, compared to the polymer without additives (355.09.012). Again the polymers 355.09.004; 355.09.006; SK13; SM14 and SM20 perform best against bacterial attachment.

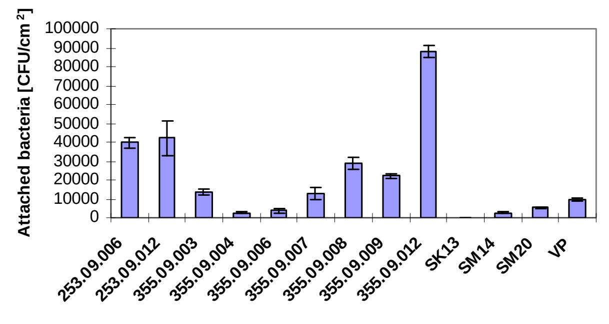

Fig.1 Bacterial attachment with *Escherichia coli*

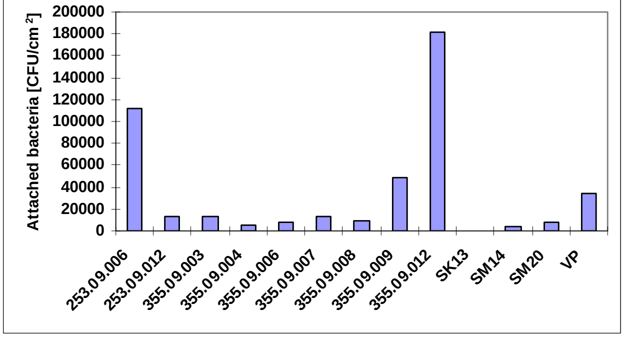

Fig.2 Bacterial attachment with *Staphylococcus aureus*

Anti-microbial efficiency of Ni-P-PTFE-biocide polymer coatings

Two types of biocide polymers SM14 and SM20 were selected and incorporated into Ni-P and Ni-P-PTFE matrix. The chemical structures of SM14 and SM20 are shown in Fig. 3. As a result, four new coatings: Ni-P-SM14, Ni-P-PTFE-SM14, Ni-P-SM20 and Ni-P-PTFE-SM20 were produced and evaluated.

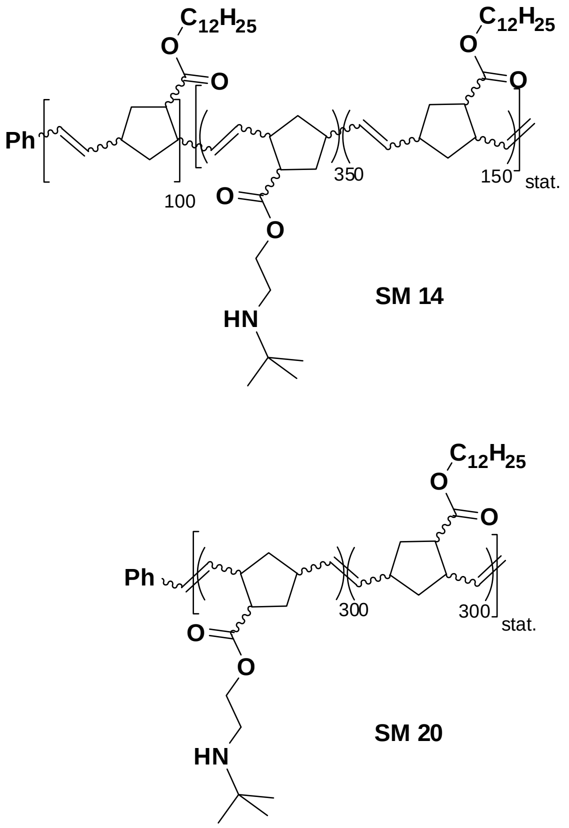

Fig. 3 Chemical structures of SM14 and SM20

Figure 4 shows *Escherichia coli* adhesion on the coatings. Stainless steel and Ni-P coatings were used as control. Overall all the coatings with biocide polymers and PTFE performed much better than stainless steel and Ni-P coatings. The coatings with biocide polymers (Ni-P-SM14 and Ni-P-SM20) performed much better than Ni-P coatings, and the coatings with PTFE (Ni-P-PTFE-SM14 and Ni-P-PTFE-SM20) performed better than the coatings without PTFE (Ni-P-SM14 and Ni-P-SM20). Ni-P-PTFE-SM14 and Ni-P-PTFE-SM20 performed best, which reduced bacterial attachment by 92%, as compared with stainless steel.

Figure 5 shows *Staphylococcus aureus* adhesion on the coatings. Similar results were obtained. Ni-P-PTFE-SM14 and Ni-P-PTFE-SM20 performed best, which reduced bacterial attachment by 86%, as compared with stainless steel.

Table 1 shows the surface energy components of Ni-P-PTFE coatings.

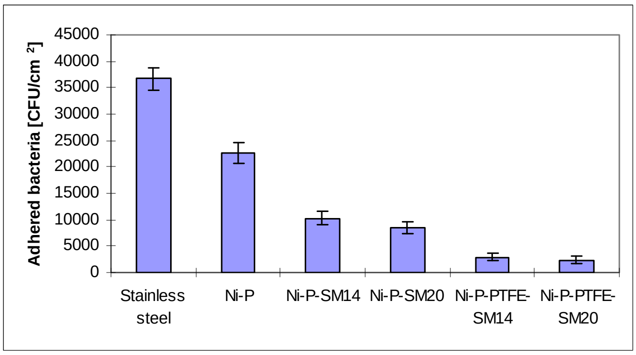

Fig 4 *Escherichia coli* adhesion on the samples for 1 hour

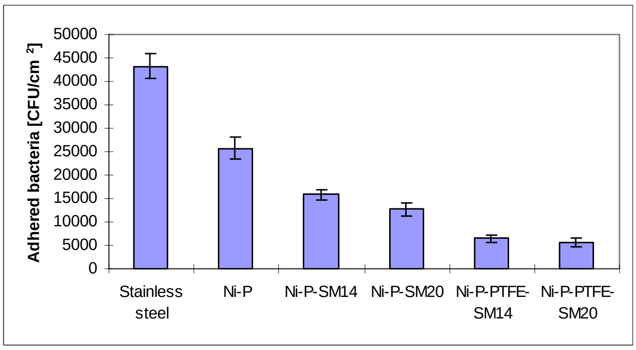

Fig 5 *Staphylococcus aureus* adhesion on the samples for 1 hour

Table 1 Surface energy components of coatings

Materials	CQ ratio	Surface Free Energy (mN/m^2)			
		γ^{LW}	γ^{+}	γ^{-}	γ^{TOT}
Stainless steel	6.76	42.58	0.04	6.32	43.56
Ni-P	3.53	31.79	0.07	9.01	33.34
Ni-P-PTFE 1	2.60	28.92	0.00	11.06	28.92
Ni-P-PTFE 2	2.32	25.85	0.00	11.12	25.85
Ni-P-PTFE 3	2.11	23.66	0.00	11.21	23.68
Ni-P-PTFE 4	1.85	20.91	0.00	11.29	20.91

The numbers of adhered bacteria (*P.fluorescens*, *Cobetia* and *Vibrio*) on the substrates (stainless steel, Ni-P coatings and Ni-P-PTFE coatings with different surface energies) were measured under static conditions at different contact times. Figures 6, 7 and 8 clearly showed that the number of adhered bacteria had strong correlation with CQ ratio (R^2 is in the range of 0.87~0.99). The number of adhered bacteria increases with increasing CQ ratio. The detailed explanation was given by Liu and Zhao (2011). The results also showed that contact time (6h, 12h and 24h) had significant influence on bacterial adhesion. For *P. fluorescens* adhesion assays (Fig.6), bacteria were suspended in phosphate buffer saline (PBS) without nutrient (TSB). While for the adhesion assays of *Cobetia* (Fig. 7) and *Vibrio* (Fig.8) they grew in SSP growth medium. In Figure 6, when contact time was 12hr, bacterial attachment was higher than at 6hr and 24hr. With time increasing, the activity of *P. fluorescens* might decrease and some could be dead due to lack of nutrient leading to a reduction of bacterial attachment. In Figures 7 and 8, the number of adhered bacteria increased with increasing contact time as enough nutrients were in the bacterial suspension. Clearly, more *Vibrio* bacteria attached to the substrates, compared with *P. fluorescens* and *Cobetia*.

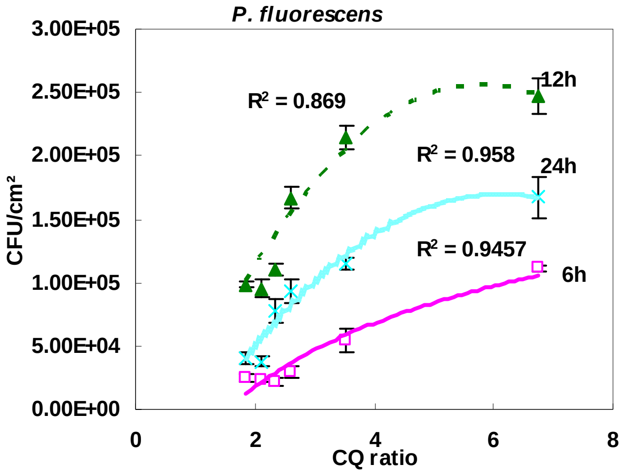

Fig. 6 Effect of CQ ratio on *P.fluorescens* adhesion

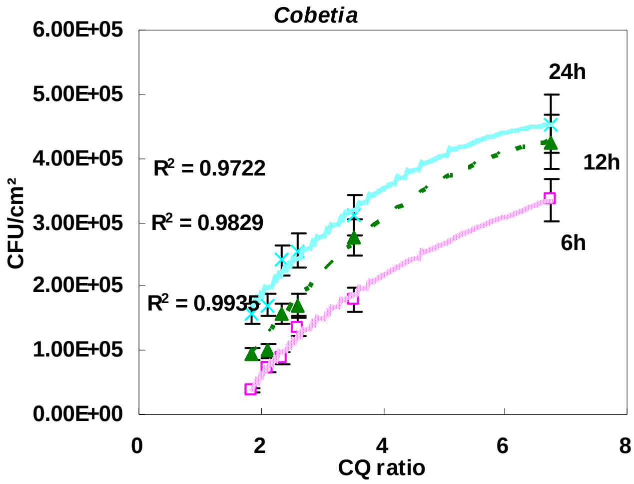

Fig. 7 Effect of CQ ratio on *Cobetia* adhesion

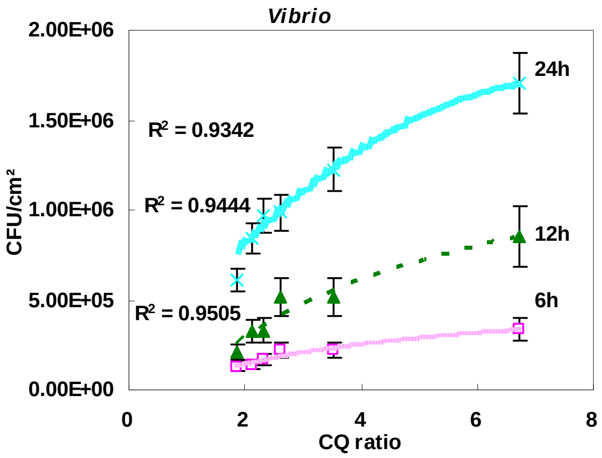

Fig. 8 Effect of CQ ratio on *Vibrio* adhesion

CONCLUSIONS

- The polymers 355.09.004; 355.09.006; SK13; SM14 and SM20 perform better than others against bacterial attachment.
- Ni-P-PTFE-SM14 and Ni-P-PTFE-SM20 performed best, which reduced bacterial attachment by 86~92%, as compared with stainless steel.
- that the number of adhered bacteria had strong correlation with CQ ratio.
- The number of adhered bacteria increases with increasing CQ ratio.

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