Antifouling properties of zinc oxide nanorod coatings

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In laboratory experiments, the antifouling (AF) properties of zinc oxide (ZnO) nanorod coatings were investigated using the marine bacterium Acinetobacter sp. AZ4C, larvae of the bryozoan Bugula neritina and the microalga Tetraselmis sp. ZnO nanorod coatings were fabricated on microscope glass substrata by a simple hydrothermal technique using two different molar concentrations (5 and 10 mM) of zinc precursors. These coatings were tested for 5 h under artificial sunlight (1060 W m$^{-2}$ or 530 W m$^{-2}$) and in the dark (no irradiation). In the presence of light, both the ZnO nanorod coatings significantly reduced the density of Acinetobacter sp. AZ4C and Tetraselmis sp. in comparison to the control (microscope glass substratum without a ZnO coating). High mortality and low settlement of B. neritina larvae was observed on ZnO nanorod coatings subjected to light irradiation. In darkness, neither mortality nor enhanced settlement of larvae was observed. Larvae of B. neritina were not affected by Zn$^{2+}$ ions. The AF effect of the ZnO nanorod coatings was thus attributed to the reactive oxygen species (ROS) produced by photocatalysis. It was concluded that ZnO nanorod coatings effectively prevented marine micro and macrofouling in static conditions.

Keywords: antifouling; zinc oxide; nanotechnology; bacteria; bryozoa; photocatalysis

Introduction

Biofouling is the undesirable accumulation and growth of marine micro and macroorganisms on submerged surfaces (Yebra et al. 2004; Eguía & Trueba 2008). It typically begins with the adhesion of microorganisms, such as bacteria and microalgae, followed by the attachment of larvae and spores of macroorganisms, eg bryozoans, barnacles, bivalves and algae (Wahl 1989). Biofouling causes severe problems in the marine industries (Townsin 2003; Yebra et al. 2004; Schultz et al. 2011) leading to metal corrosion, affecting the performance of equipment and installations in seawater, such as pipelines, cooling system units, seawater intakes and reverse osmosis membranes of desalination plants (Terlizzi et al. 2001; Schultz 2007; Bianco et al. 2009). Additionally, biofouling on ship and boat hulls results in reduced fuel efficiency and a lowering of cruising speed, which lead to increased carbon dioxide emissions (Yebra et al. 2004; Schultz et al. 2011). Thus, the eradication and prevention of biofouling are very important for the maritime industries.

Common techniques used for protection from biofouling include the use of biocides (Yebra et al. 2004; Deauney et al. 2010; Thomas & Brooks 2010). However, biocides cause a significant impact on non-target organisms (Thomas & Brooks 2010), as often high concentrations are required to eradicate fouling, leading to extensive environmental pollution (Morton et al. 1998; Inbakandan et al. 2013).

Due to the problems accompanying current antifouling (AF) approaches, numerous efforts have been made to develop novel, less toxic yet effective techniques for the prevention of biofouling (Finnie & Williams 2010; Callow & Callow 2011; Dobretsov et al. 2013). Some attempts have been made towards fabrication of AF coatings by the modification of surface properties (Schmelmer et al. 2007; Beigbeder et al. 2008; Martinelli et al. 2008, 2012). It has been proposed that the physico-chemical properties of nanostructured coatings prevent biofouling (Callow & Callow 2011). For example, silver nanoparticles can prevent biofilm formation by Pseudomonas aeruginosa (Zhang et al. 2014). It was shown that metal oxide nanolayers can prevent the growth of some microorganisms, eg bacteria and fungi, through a photocatalytic process (Yang et al. 2013). This process is based on the generation of reactive oxygen species (ROS) and the slow release of toxic metal ions when metal oxide nanomaterials were illuminated with light of a certain wavelength (Miller et al. 2010). Titanium dioxide (TiO$_2$), silver oxide (AgO), carbon nanotubes and zinc oxide (ZnO) nanoparticles and nanorods have been used as antimicrobial agents for disinfection of freshwater and in biomedical applications (Sapkota et al. 2011; Baruah et al. 2012; Carl et al. 2012; Kubacka et al. 2013; Yang et al. 2013).

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ZnO nanoparticles and nanorod coatings are attractive due to an easy and controlled method of fabrication through a simple sol-gel and hydrothermal process (Baruah & Dutta 2009; Sapkota et al. 2011; Baruah et al. 2012). Supported ZnO nanorod coatings have significant advantages over ZnO nanoparticles because of the increased stability, lower toxicity and reduced loss of nanoparticles (Li et al. 2011; Jansson et al. 2012). Another noticeable advantage of ZnO nanorod coatings includes the possibility of using a large variety of support materials and a low temperature synthesis process (<100°C) (Baruah et al. 2009). It has been shown that ZnO nanorod coatings inhibited the growth of bacterial pathogens including Escherichia coli, Bacillus spp., Salmonella spp., Listeria monocytogenes and Staphylococcus aureus in freshwater (Jones et al. 2008; Liu et al. 2009; Jaisai et al. 2012).

The main purpose of this study was to investigate the AF effect of ZnO nanorod coatings against the marine bacterium Acinetobacter sp. AZ4C, the microalga Tetraselmis sp. and larvae of the macrofouling bryozoan Bugula neritina in laboratory experiments.

Materials and methods

ZnO nanorod coatings were prepared by seeding surfaces of clean glass microscope slides with ZnO nanocrystal-lites followed by the hydrothermal growth of ZnO nanorods. Microscope glass substrata were cleaned by following standard procedure. Briefly, the glass slides were sonicated in soap solution for 30 min and then thoroughly rinsed with deionized water until soap residues were removed. The glass slides were then sonicated in methanol for 30 min followed by sonicating in acetone for 30 min to remove organic residues. The glass slides were dried and stored in a desiccator until used.

ZnO nanocrystallite seeding

A 10 mM solution of zinc acetate dihydrate (Zn(CH$_3$COO)$_2$·2H$_2$O; Merck, Germany) in 25 ml of deionized water (DI) was used as the seeding solution, which was sprayed on pre-heated (350°C) substrata (Mahmood & Dutta 2011; Mahmood et al. 2013). A total volume of 25 ml of seeding solution was sprayed onto each microscope glass substratum prior to the hydrothermal growth of ZnO nanorods. After spraying, the samples were annealed in an atmospheric furnace at 350°C for 5 h. The samples were stored in an oven at 95°C until they were used in experiments.

Hydrothermal synthesis of ZnO nanorods

ZnO nanorods were grown on the pre-seeded microscope glass substrata following a hydrothermal route described previously (Baruah & Dutta 2009). Briefly, the seeded substrata were placed in a chemical bath containing equi-molar concentrations of zinc nitrate hexahydrate (Zn(NO$_3$)$_2$·6H$_2$O; APS Ajax Finechem, Sydney, Australia) and hexamethylenetetramine ((CH$_2$)$_6$N$_4$; Sigma-Aldrich, St Louis, MO, USA) (precursor solution) and kept in a preheated oven at 90°C. Two different concentrations of precursor solution (5 mM: sample I and 10 mM: sample II) were used for the hydrothermal synthesis of ZnO nanorods. ZnO nanorod growth was carried out for 10 h and the precursor solution was replenished after the first 5 h of growth (Baruah & Dutta 2009). After the growth of the ZnO nanorods, the samples were carefully and thoroughly rinsed with DI water. To test the effect of annealing temperature on the electronic defects in the ZnO nanorod coatings, sample II was annealed in an atmospheric furnace for 1 h at a range of temperatures (100, 150, 250, 350 and 450°C). Finally, samples I and II annealed at 250°C were selected for photocatalysis experiments (see below).

Characterization of ZnO nanorod coatings

The surface morphology of ZnO nanorods on glass substrata (samples I and II) with and without a biofilm of the bacterium Acinetobacter sp. AZ4C (see below) was characterized using a JEOL JSM-6301F (Japan) field emission scanning electron microscope (FESEM) working at 20 kV. ZnO crystal structures were studied by using X-ray diffraction (XRD) (Rigaku Miniflex 600, Tokyo, Japan) working at 40 kV, 15 mA. The spectra were collected at 10° min$^{-1}$ at each 0.02° interval, for 20 values from 3 to 90°. A Perkin-Elmer fluorescence spectrometer (LS 55, Santa Clara, CA, USA) was used to study the defects in the ZnO materials following annealing, using an excitation wavelength of 350 nm. Optical absorption was measured at 620 nm using a plate reader (Thermo Scientific, Waltham, MA, USA). X-ray photoemission spectroscopy (XPS) (Omicron Nanotechnology, Taunusstein, Germany) with monochromatic Al K$_\alpha$ radiation ($h\nu = 1486.6$ eV) working at 15 kV was used for surface characterization of the ZnO nanorod samples. The binding energies were calibrated with respect to the C 1s feature at 284.6 eV. Inductively coupled plasma optical emission spectroscopy (ICP-OES: Varian 710 ES, Santa Clara, CA, USA) was used to determine the dissociated Zn ions from ZnO nanorod coatings in seawater under light and dark conditions. A solar simulator (Model SS 1.6 kW, Sciencetech Inc., London, ON, Canada) generating air mass 1.5 (AM 1.5) illumination of solar spectra was used to conduct photocatalysis experiments. Three sets of measurements were carried out and average values are reported. Water contact angles using a sessile drop (5 $\mu$l of deionized water) were measured at five different locations on each ZnO nanorod coated substratum with a Theta Lite attention tensiometer (Biolin Scientific, Stockholm, Sweden) and the average values are reported.
Dissolution of ZnO nanorods in seawater

ZnO nanorod coatings (sample II with an area of ~ 3.5 cm²) were dipped individually in 4 ml of sterile seawater in cuvettes. The cuvettes were either placed under visible light (irradiation ~ 1,060 W m⁻²) generated by the solar simulator or under a dark environment covered with aluminum foil. This experiment was carried out for 5 h.

Investigation of AF activity

The effect of ZnO nanorod coated glass substrata (area = 1 cm²) synthesized with 5 mM (sample I) and 10 mM (sample II) precursor solutions on the marine bacterium Acinetobacter sp. AZ4C, larvae of the bryozoan B. neritina and the microalga Tetraselmis sp. was studied. 24-well plates (Costar, Tewksbury, MA, USA) were used and the area of glass sample substratum coated with ZnO nanorod coatings was 1 cm² for both samples (I and II). All experiments were conducted in triplicate. Sterile clean microscope glass slides (area = 1 cm²) were used as controls. Two similar sets of plates were prepared, one of which was exposed to visible light (~ AM1.5 irradiation ~ 1,060 W m⁻² and 530 W m⁻²). During antibacterial experiments, an irradiation of 1,060 W m⁻² was used, while in the antialgal and antilarval experiments the light intensity was reduced to 530 W m⁻² due to the high mortality of algal cells and larvae in the control treatments (without ZnO nanorod coatings) when an irradiation of 1,060 W m⁻² was employed. A second set was covered with aluminum foil and used as a dark treatment (no irradiation). Each experiment was conducted for 5 h at 35°C.

Antibacterial experiments

The marine bacterium Acinetobacter sp. AZ4C (99% similar to Acinetobacter sp. GenBank: Z93451.1) was isolated from 21 d old biofilms developed on an artificial substratum exposed to seawater in Marina Bandar Rowdah (Muscat, Oman 23° 34’ 55” N 58° 36’ 27” E). A stock of this bacterium was maintained at ~80°C. Prior to the experiments, the bacterium was cultured in Marine Broth (Difco, Bergen County, NJ, USA) for 12 h at 30°C. The suspension was centrifuged at 5,000 g and re-suspended in sterile Marine Broth to obtain an absorbance at 620 nm from 0.123 ± 0.003. Each of the 24-well plates containing nanorod coatings or controls (bare glass) was filled with 1.5 ml of the bacterial culture prior to the experiment. Experiments were conducted at 35°C. After 5 h, bacteria from the experiment were used to determine growth, total DNA, biofilm formation and for scanning electron microscopy (SEM).

Inhibition of bacterial growth

At the end of the antibacterial experiments, 100 μl of the broth culture from each well (both under light and dark conditions) were collected and the absorbance was measured at 620 nm using a plate reader (Thermo Scientific). Three readings were taken and the mean value was calculated.

DNA concentration

For DNA isolation, 200 μl of bacterial broth culture from antibacterial experiments (both under light and dark conditions) were transferred into sterile Eppendorf® tubes and centrifuged at 13,000 rpm for 5 min. After discarding the supernatant, 100 μl of autoclaved Nuclease-free water (Ambion, Grand Island, NY, USA) were added to each tube and cell pellets were dispersed using a vortex mixer. Subsequently, the mixture was placed in boiling water for 7 min then centrifuged at 13,000 rpm for 5 min. Finally, the upper layer containing bacterial DNA was collected and DNA concentrations were measured using Nanodrop 2000C (Thermo Scientific). From triplicate measurements, the mean concentration was calculated.

Biofilm formation

At the end of the antibacterial experiments, filmed coatings and controls (bare glass) were washed with sterile seawater and attached cells were stained with a 1% (w/v) solution of crystal violet in water at ambient temperature (25°C) for 15 min in the dark. The substratum was washed three times with sterile water and then dried at room temperature. The samples were transferred into a new multiwell plate and the dye was dissolved with ethanol. The absorbance at 620 nm was measured using a plate reader (Thermo Scientific). Since the nanorod coatings (samples I and II) were able to absorb about 8% of the dye, coatings without biofilm were stained with dye. The absorbance from clean coatings was subtracted from the readings of coatings with biofilms. Three replicate readings were taken and the mean absorbance was calculated.

Antilarval experiments

Larvae of the bryozoan B. neritina were obtained from pontoons in Marina Bandar Rowdah (Muscat, Oman) and collected as previously reported (Dobretsov & Qian 2006). Prior to the experiments, 900 μl of sterile seawater were added to each well with ZnO nanorod coatings (samples I and II) or the control (bare glass). Seawater (100 μl) containing 10–20 B. neritina larvae was added into each well. After 5 h, the number of dead larvae, attached larvae and the total number of larvae in each well were counted using a dissecting microscope (Zeiss, Oberkochen, Germany). Additionally, the toxicity of Zn²⁺ ions against larvae was investigated using zinc chloride (ZnCl₂) (Sigma-Aldrich) solutions in filtered
seawater. In this experiment, 10–20 B. neritina larvae were added to each well containing 1 ml 400 µg l−1 ZnCl₂ solution or seawater (control). After 5 h, the number of dead larvae, attached larvae and the total number of larvae in each well was counted using a dissecting microscope. The percentage mortality (number of dead larvae/total number of larvae × 100) and settlement (number of settled larvae/total number × 100) were calculated from three replicates and the mean percentage mortality is reported.

**Antialgal experiments**

In these experiments, 1 ml of *Tetraselmis* sp. culture (absorption at 620 nm = 0.161 ± 0.001) was placed into each well of the plate containing ZnO nanorod coatings (samples I and II) or controls (bare glass). After 5 h, 100 µl of the culture were taken from each well and the absorbance was measured at 620 nm using a plate reader (Thermo Scientific). Three replicate readings were carried out and the mean absorbance was calculated.

**Statistical analysis**

The data from antibacterial, antilarval and antialgal assays were square root transformed in order to ensure normality of variance. The normality data assumption was verified with the Shapiro–Wilk’s test (Shapiro & Wilk 1965). The effect of coating type and presence of light on the bacteria, the larvae and the microalgal cells was investigated by two-way analysis of variance (ANOVA). Differences between the control and the treatments were determined by ANOVA followed by Dunnett’s post hoc test. All calculations were performed using Statistica version 11.0 (StatSoft, Tulsa, OK, USA) software. In all cases, the threshold for significance was 5%.

**Results**

**Characterization of ZnO nanorod coatings**

Scanning electron micrographs (SEM) of nanorod coatings (samples I and II) on a glass substratum are shown in Figure 1a–d. Uniform growth of ZnO nanorods was observed with an average width and length of 100 nm and 2.3 µm respectively for sample I, and 120 nm and 3.5 µm respectively for sample II. Figure 1e shows an X-ray diffraction (XRD) pattern of ZnO nanorods grown on glass substrata (samples I and II). Preferential growth along the (002) direction and the hexagonal wurtzite structure of ZnO nanorods (See Figure 1b–d inset) was confirmed by XRD measurements with 20 values of 31.8, 34.4, 36.2, 47.4, 62.9 and 72.7 (JCPDS card no. 36-1451) corresponding to (100), (002), (101), (102), (103) and (104) crystal planes respectively (Baruah & Dutta 2009; Myint & Dutta 2012).

Table 1 shows the calculated and estimated profiles of samples I and II. The total number of ZnO nanorods on the sample substrata (1.18 × 10⁹ and 1.037 × 10⁹ for samples I and II, respectively), amount of catalyst (~120 µg for sample I and 190 µg for sample II) and effective area of ZnO nanorod coated surfaces (sample I = 27 cm² and sample II = 26 cm²) were estimated (details of the estimated surface area is given in Figure S1; Supplementary information is available via a multimedia link on the online article webpage). The surface coverage of ZnO nanorods on the substrata was found to be 9% for sample I and 9.4% for sample II (Figure S2) and was confirmed by measuring the surface wetting of samples I and II (Figure S3).

Figure 2a–c shows the XPS spectra of ZnO nanorod coated glass substrata (overall scan), Zn 2p and O 1s peaks for samples I and II, respectively. The photoluminescence (PL) spectra of ZnO nanorods on a glass substratum (sample II) annealed at different temperatures (90, 150, 250, 350, 450 and 550°C) showed the transition peaks in UV region at ~ 375 nm and in the visible region between 500 nm to 650 nm (Figure 3). The maximum PL intensity was observed for ZnO samples annealed at 250°C and the lowest intensity was observed from samples annealed at 550°C.

**Dissolution of Zn²⁺ ions in seawater**

Since ZnO is amphoteric, the dissociation of ZnO in seawater was observed under visible light irradiation (1,060 W m⁻²) and in the dark (no irradiation) (Figure 4). Under visible light irradiation the highest concentration of Zn²⁺ ions was found to dissociate into the aqueous medium.

**Antibacterial experiments**

After 5 h, the absorbance of the marine bacterium *Acinetobacter* sp. AZ4C culture was different (Figure 5a). Two-way ANOVA showed that both treatments (ANOVA: F₂ = 56.8; p < 0.0001) and illumination (ANOVA: F₁ = 85.7, p < 0.0001) affected the absorbance of the bacterial culture. Under light irradiation in the presence of ZnO nanorods, a significant reduction in absorbance (Dunnett’s test, p < 0.05) was observed compared to the control (Figure 5a).

Both treatments (ANOVA: F₂ = 175.7, p < 0.0001), illumination (ANOVA: F₁ = 128.1, p < 0.0001), and their combination (ANOVA: F₂ = 49.6, p = 0.0001) affected the concentration of DNA extracted from *Acinetobacter* sp. AZ4C cultures (Figure 5b). Concentrations of DNA were significantly lower (Dunnett’s test, p < 0.05) in the presence of sample I and sample II in both darkness and under light irradiation. The DNA concentration in the control was higher in darkness (Figure 5b).
Figure 1. SEM images of hydrothermally synthesized ZnO nanorods grown on a glass substratum. (a) Cross sectional view (sample I); (b) top view (inset: magnified image of the hexagonal structure of a single crystal ZnO nanorod showing the diameter of the nanorod) (sample I); (c) cross sectional view (sample II); (d) top view (inset: magnified image of the hexagonal structure of a single crystal ZnO nanorod showing the diameter of the nanorod) (sample II); (e) X-ray diffraction pattern of the hexagonal wurtzite structure ZnO nanorod coated glass substrata (Samples I and II, JCPDS card no. 36–1451).
After 5 h, biofilm formation by *Acinetobacter* sp. AZ4C was different (Figure 5c). Two-way ANOVA showed that both treatments (ANOVA: $F_2 = 149.2; p < 0.0001$), illumination (ANOVA: $F_1 = 431.7; p < 0.0001$) and their combination (ANOVA: $F_2 = 144.8; p < 0.0001$) affected biofilm formation. Biofilm formation was similar in darkness for all the tested coatings, while both samples I and II significantly reduced biofilm formation in light (Dunnett’s test, $p < 0.05$).

SEM micrographs showed both ZnO nanorod sample I and II coatings caused a decrease in the number of attached bacteria in comparison to the control kept in the dark (Figure S4). No cell lysis was observed. There was no significant difference between the biofilms that developed in the light and in control samples kept in the dark (data not shown).

### Table 1. Estimated and theoretical calculation of sample profiles (sample I: 5 mM and sample II: 10 mM).

| ZnO nanorods | Sample area of 1 cm² | No. of ZnO rods | Amount of ZnO (mg) | Effective surface area (cm²) |
|--------------|----------------------|-----------------|--------------------|-----------------------------|
| Sample I (5 mM) | 100 | 2.3 | $1.18 \times 10^9$ | 0.12 | 27.1 |
| Sample II (10 mM) | 120 | 3.5 | $1.04 \times 10^9$ | 0.19 | 26.2 |

Figure 2. (a) XPS spectra of hydrothermally grown ZnO nanorod coated glass substrata, samples I and II; (b) Zn 2p peak (samples I and II); (c) O 1s peak (samples I and II).

Figure 3. Photoluminescence spectra of hydrothermally grown ZnO nanorods on glass substrata (sample II) (350 nm excitation) upon annealing for 1 h at different temperatures.

Figure 4. The dissolution of ZnO nanorods (sample II) in seawater as a function of an exposure time of 5 h under visible light irradiation (1,060 W m⁻²) and in the dark (no irradiation).
Antilarval experiments

Illumination (ANOVA: $F_1 = 247.2$, $p < 0.0001$), treatments (ANOVA: $F_2 = 79.7$, $p < 0.0001$) and their combination (ANOVA: $F_2 = 53.8$, $p < 0.0001$) significantly affected the mortality of $B. neritina$ (Figure 6a). Both samples significantly (Dunnett test, $p < 0.05$) increased the mortality of larvae in samples exposed to light, which were ~23–25-fold higher for samples I and II, compared to the control. In darkness, no significant (Dunnett’s test, $p > 0.05$) mortality of larvae was observed (Figure 6a).

**Antilarval experiments**

Illumination (ANOVA: $F_1 = 247.2$, $p < 0.0001$), treatments (ANOVA: $F_2 = 79.7$, $p < 0.0001$) and their combination (ANOVA: $F_2 = 53.8$, $p < 0.0001$) significantly affected...
Figure 7. The effect of ZnO nanorod coatings in light (530 W m\(^{-2}\)) or dark (no irradiation) conditions on the growth of *Tetraselmis* sp. after 5 h. ZnO nanorods were synthesized with 5 mM (sample I) and 10 mM (sample II) precursor solutions. The control was bare glass. * = significantly different (\(p<0.05\)) data according to Dunnett’s *post hoc* test. Data are the means ± SDs of three replicates.

settlement of *B. neritina* (Figure 6b). Under light conditions, low settlement (Dunnett’s test, \(p<0.05\)) of larvae was observed on samples I and II. In darkness, high settlement of larvae was observed on all tested samples (Figure 6b).

Experiments with ZnCl\(_2\) solutions demonstrated that Zn\(^{2+}\) ions at 400 μg l\(^{-1}\) were not toxic to larvae (data not shown). After 5 h, settlement of larvae in the presence of Zn\(^{2+}\) ions (100 ± 0%) was not different from the control (97.5 ± 5%).

**Antialgal experiments**

Illumination (ANOVA: \(F_1 = 1463.5\); \(p<0.0001\)), treatments (ANOVA: \(F_2 = 186.4\), \(p<0.0001\)), and their combination (ANOVA: \(F_2 = 187.0\), \(p<0.0001\)) affected the densities of the marine microalga *Tetraselmis* sp. (Figure 7). Under light conditions, both samples significantly (Dunnett’s test, \(p<0.05\)) decreased the microalgal density. In darkness, there were no significant differences (Dunnett test, \(p>0.05\)) between the densities of algal cells subjected to different treatments (Figure 7).

**Discussion**

The estimation and calculation of the effective surface area of ZnO nanorod coatings (samples I and II), nanorod surface coverage (%) and the effective surface area were found to be very similar (Table 1). The diameter and height of nanorods were, however, different. The nanorods of sample II (prepared with 10 mM precursor solution) were longer than those in sample I (prepared with 5 mM precursor solution), which resulted in a slightly higher surface hydrophobicity of sample II (Myint et al. 2011, 2012).

The structure of the ZnO nanorods was confirmed by XRD (crystal structure) (Figure 1e) and the binding energy difference (due to the spin orbit splitting) in the Zn 2p peak from XPS analysis (Figure 2b) (Byrne et al. 2010). Single crystalline ZnO nanorods were found to preferentially grow along the (002) axis of the hexagonal wurtzite structure as confirmed with standard XRD data (JCPDS card no. 36-1451). From XPS, an overall scan of ZnO nanorod coated glass substrata (Figure 2a), Zn 2p and O 1s peaks were found, but no extra peak, other than zinc and oxygen, corresponding to any impurities that could be observed on the surface of either sample. The carbon peak with a binding energy of 284.6 eV arises from the adventitious carbon on the ZnO material surface (Sardar et al. 2013). Zn 2p peaks of 1021.6 eV (Zn 2p\(_{3/2}\)) and 1044.8 eV (Zn 2p\(_{1/2}\)) with the binding energy difference of 23.2 eV (see Figure 2b) indicate the normal state of Zn\(^{2+}\) in ZnO nanorods (Mu et al. 2011). The O 1s signal feature comprises three distinct peaks corresponding to O–Zn, O–H and H\(_2\)O respectively (Ogata et al. 2004) (Figure S5).

Photoluminescence spectra of ZnO nanorods show the presence of two main noticeable peaks attributed to radiative recombinations (band-to-band) and broad band transitions (Figure 3). The broad peak in the visible region between 500 nm to 650 nm is attributed to the recombinations through defect states in ZnO such as zinc interstitials (Z\(_{ni}\)), oxygen vacancies (V\(_o\)) and zinc vacancies (V\(_{Zn}\)), respectively (Mahmood et al. 2011; Swati et al. 2013). The surface defects on the ZnO nanorods arising from oxygen non-stoichiometry increases upon annealing at temperatures up to 250°C. In the samples annealed at 350°C to 500°C, the eventual saturation of the surface defects occurs as observed from the lowering of the visible emission. Thus, the temperature of 250°C was selected for annealing of the samples prior to photocatalysis.

The present study has clearly demonstrated that under light irradiation, in the presence of ZnO nanorod coatings, absorbance of a culture of the marine bacterium *Acinetobacter* sp. AZ4C was lower compared to the control (Figure 5a). This suggests that the density of cells in the presence of nanorods under light irradiation was lower. Within these experimental conditions, bacteria should be able not only to divide but also to attach to the wells and the nanorod coatings. Since a low bacterial density was found in the aqueous medium only upon light irradiation, it can be reasonably concluded that the reduction observed was due to the inhibitory effect of photocatalysis in the presence of ZnO nanorods. Previous studies demonstrated that ZnO nanorods inhibited the growth of Gram-positive (*Staphylococcus aureus*,...
S. epidermis, Bacillus subtilis, B. atrophaeus) and Gram-negative (Escherichia coli, Aerobacter aerogenes, P. aeruginosa) bacterial pathogens in the presence of light (Jones et al. 2008; Liu et al. 2009; Jaisai et al. 2012; Mahmood et al. 2012). Similarly, ZnO nanoparticles showed strong antimicrobial activity against bacterial pathogens, such as B. atrophaeus, S. aureus, S. epidermis, and S. agalactiae (Jones et al. 2008; Liu et al. 2009). ZnO nanoparticles increased the expression levels of oxidative stress and general stress response genes in the bacterium Campylobacter jejuni (Xie et al. 2011). The present study is the first to demonstrate the antibacterial properties of ZnO nanorod coatings against a marine fouling bacterium.

ZnO nanorod coatings inhibited biofilm formation by Acinetobacter sp. AZ4C by 2.3–3.5-fold in comparison to the control (bare glass) under light irradiation (Figure 5c). Bacteria on the nanorod-coated surfaces exposed to light formed fewer clumps in comparison with bacteria on the control surfaces as observed by electron microscopy (Figure S4). Previously it was shown that ZnO nanorods are capable of modulating the adhesion and viability of macrophage (Zaveri et al. 2010) and bacterial cells (Jansson et al. 2012). Since the roughness of the nanostructured surface remains the same it is unlikely that roughness affected attachment of bacteria in the current study. Previously, it has been shown that exposure of bacterial pathogens to ZnO nanorod coatings under light irradiation damaged cells and changed their morphology (Jain et al. 2013). However, in this study, no changes in bacterial cell morphology were observed (Figure S4). Differences in the results could be due to differences in the ZnO coatings and the bacterial species used in the respective studies.

ZnO nanorod coatings under light irradiation showed high mortality and lower settlement of larvae of the bryozoan B. neritina (Figure 6). In contrast, larval settlement on ZnO nanorod coatings was not different from the control in the dark. It has been shown that ZnO nanoparticles at concentrations of 1–100 mg l\(^{-1}\) caused significant mortality of zebra fish embryos, retarded zebra fish hatching, reduced the size of the fish and increased abnormalities (Bai et al. 2010). Similarly, ZnO nanoparticles at a concentration of 3.2 mg l\(^{-1}\) were found to be toxic to the crustacean Daphnia magna (Heimlaan et al. 2008). This study is the first to demonstrate that ZnO nanorod coatings inhibit larval settlement.

ZnO nanoparticles have been found to be toxic to the fresh water microalga Pseudokirchneriella subcapitata under visible light illumination (Franklin et al. 2007). ZnO nanoparticles under low UV radiation were found to be responsible for the reduction in density of the green microalga Stichococcus sp. (Gladis et al. 2010), which is in good agreement with the results of this study, which showed that ZnO nanorod coatings inhibited the density of the marine microalga Tetraselmis sp. under sunlight (Figure 7).

This study proposed that two types of sample (I and II, prepared with 5 mM and 10 mM zinc precursor solutions) would result in the formation of ZnO nanorods of different diameter and height, thereby affecting bioactivity (Bucaro et al. 2012). It has been reported that the antibacterial activity of ZnO nanoparticles was directly proportional to the concentration and size of the nanoparticles (Siddique et al. 2013). The dimensions of the ZnO nanorods, surface roughness and nanorod density (number of rods per unit area) of samples I and II were quite similar in this study, which may explain why no significant difference in AF activity was found between these samples.

The AF mechanism of ZnO nanorod coatings and particles with microorganisms remain unclear (Li et al. 2011; Xie et al. 2011). Based on analysis of the literature, three different mechanisms of the AF action of ZnO nanorods are proposed: (1) the micro/nano-topography of the coatings; (2) the toxicity of Zn\(^{2+}\) ions released from the coating; and (3) the production of reactive oxygen species (ROS) under an appropriate environment. It has been shown that micro-textured surfaces are able to prevent the settlement of fouling organisms (Natalio et al. 2012; Vucko et al. 2013). In the laboratory, all micro-textured surfaces prevented attachment of the diatoms Nitzschia closterium and Amphora sp., while settlement of zoospores of Ulva sp. and larvae of B. neritina was found to be lower on substrata with a surface micro-topography that was slightly smaller than the propagule size (Vucko et al. 2013). Even though the settlement of larvae of B. neritina would be affected by surface topography at the nanoscale level, this does not account for the significant differences observed in the AF performances of ZnO nanorod coatings exposed to light and in the dark.

It is well known that ZnO nanoparticles and nanorods slowly dissociate in water and release Zn\(^{2+}\) ions (Han et al. 2010). Release of Zn\(^{2+}\) ions from ZnO nanorod surfaces depends on the area of the exposed surface, crystal defects, the surface energy of the nanorods, the pH and ionic environment and dissolved organic matter (Li et al. 2013). The results show negligible dissolution of ZnO nanorods in seawater at a pH of ~ 7.8 for 5 h. The concentrations of Zn\(^{2+}\) ions in the water were lowest in samples kept in the dark and twofold higher in sample II irradiated with light. Dissolution of sample I synthesized at a lower molarity of precursor leads to a low concentration of zinc ions compared to sample II. Photo-corrosion leads to a faster dissociation or faster release of Zn\(^{2+}\) ions when ZnO nanorods are irradiated with light, which is favorable for the inhibition of bacterial growth (Baruah et al. 2012). Considerable toxicity of Zn\(^{2+}\) ions to bacteria at 1 mg l\(^{-1}\) has been reported (Li et al. 2011). ZnO nanoparticles were highly toxic to
bacteria (E. coli and S. aureus) at concentrations > 1–3.4 mM (81.4–276.7 mg L⁻¹), while these concentrations were not toxic to human T lymphocytes (Reddy et al. 2007). Interestingly, Zn²⁺ ions produced by ZnO nanoparticles were reported to be highly toxic to the crustacean Thamnocephalus platyurus and the bacterium Vibrio fischeri, but less toxic to the crustacean D. magna (Heinlaan et al. 2008). In this study, larvae of B. neritina were not affected by concentrations of Zn²⁺ ions similar to those achieved by dissolution of ZnO nanocoatings (sample II) under light irradiation. This suggests that the toxicity of Zn²⁺ ions released does not fully explain the AF properties of ZnO nanorod coatings.

Due to the external activation of light (photons) with the energy (hv) ≥ bandgap energy of ZnO nanomaterial, electron-hole pair generation takes place on ZnO surfaces. These generated electrons and holes react quickly with O₂ and OH⁻ that exist in water to form reactive oxygen species (ROS), such as hydroxyl radicals, hydrogen peroxide and superoxide anions. The presence of hydroxyl groups on cell membranes, causing damage to DNA (Nel et al. 2005; Mahmood et al. 2012). Additionally, ROS can enter cells through damaged cell membranes, causing damage to DNA (Nel et al. 2006; Baruah et al. 2010). In the current study, lower concentrations of DNA were observed in the bacteria collected from samples treated in the presence of light irradiated ZnO nanorod coatings (Figure 5b). A higher concentration of ROS species could lead to the deterioration of polysaccharides impacting extracellular polymeric substances and whole bacterial cells (Gladis et al. 2010). High mortality and low larval settlement, and lower densities of bacteria and microalgae, were observed only when ZnO nanorod coatings were exposed to light. This suggests that the AF effect observed was most likely due to exposure of the organisms to ROS.

In conclusion, this study has demonstrated that ZnO nanorod coatings in the presence of sunlight reduced the density of the marine bacterium Acinetobacter sp. AZ4C and the marine alga Tetraselmis sp., prevented biofilm formation and decreased settlement of bryozoan larvae under laboratory conditions. This suggests that ZnO nanorod coatings have the potential to effectively prevent micro and macrofouling in the marine environment. Longer laboratory and field experiments are needed in order to establish the AF activity of ZnO nanorod coatings prior to industrial applications.

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