



Anti-biofouling functional surfaces for marine aquaculture

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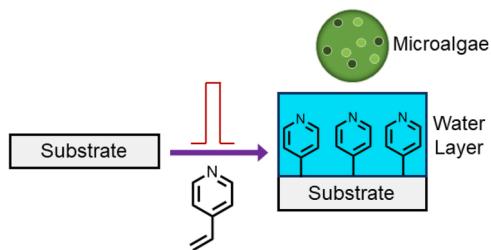
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HIGHLIGHTS

- Nanocoatings are fabricated with varying surface wetting.
- A structure–behaviour relationship correlates surface wetting to extent of biofouling during microalgae culture exposure.
- High wetting (hydrophilic) surfaces are better at reducing biofouling compared to water repelling (hydrophobic) surfaces.
- Most effective are non-toxic pulsed plasma poly(4-vinylpyridine) nanocoatings.

GRAPHICAL ABSTRACT



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ABSTRACT

Aquaculture is a global-scale industry providing sustainable production of protein-rich foods required to feed the growing world population. Microalgae cultivation in aquaculture bioreactors can exude extracellular polymeric substances leading to biofouling of culture infrastructure and increased algal disease risk. A structure–behaviour relationship was developed by examining how the surface wettability of a range of functional nanocoatings impacts the extent of biofouling during exposure to continuous microalgae culture under normal hydrodynamic conditions. High-wetting (hydrophilic) surfaces were found to reduce biofouling better than water-repelling (hydrophobic) surfaces. Low toxicity in conjunction with antifouling behaviour was found for pulsed plasma poly(4-vinylpyridine) coated bioreactor surfaces (water contact angle = $38 \pm 5^\circ$) towards the marine microalgal species *Chaetoceros calcitrans*, *Chaetoceros mulleri*, and *Tisochrysis lutea* (T-Iso), which are commonly grown as aquaculture food.

1. Introduction

Over half of the fish eaten by humans originate from aquaculture [1]. Therefore, this sustainable industry plays a key role in providing protein-rich food to the growing world population. The structures used in aquaculture production systems consist of a variety of submerged surfaces, which are susceptible to biofouling and the significant costs associated with biofouling control. Historically, paints containing tributyltin have had widespread use to mitigate marine biofouling [2].

However, serious concerns about toxicological effects on marine ecosystems have arisen over the years, and many countries have now banned their use [3]. Some reports detail the use of antimicrobial additives contained within coatings in order to prevent biofouling. One study showed chitosan polymer nanocomposites containing TiO₂/Ag are antifouling and anti-algal against the microalga *Dunaliella salina* [4]. The TiO₂ mode of antimicrobial action involves production of reactive oxygen species upon UV irradiation. In another case, polydopamine–Ag nanoparticle coatings inhibited attachment by *Dunaliella tertiolecta* [5].

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There still are concerns about the toxic effects of silver upon release into the environment, in addition to the potential for antimicrobial resistance [6,7]. Copper-doped TiSiN coatings have inhibited adhesion of *Phaeodactylum tricornutum*, *Nitzschia closterium* and *Chlorella* sp. due to the algicidal effect of leaching copper [8]. The use of non-leaching polymer coatings to mitigate biofouling by microalgae includes coatings based upon poly(styrene)-*b*-poly(ethylene glycol) methacrylate copolymer [9], UV-cured poly(isoprene)-based [10], and negatively charged poly-(styrenesulfonate)/poly-(diallyldimethylammonium chloride) layer-by-layer assemblies [11]. Such coating methods suffer from disadvantages including being multi-step, solvent-based, and often 3-dimensional objects are difficult to coat.

In this study, pulsed plasmachemical deposition has been employed with the fabrication method providing advantages of speed, single-step, dry (solventless), independent of substrate material, excellent adhesion, low energy consumption, minimal waste, and conformal coating. Mechanistically, this approach entails two distinct reaction regimes: the short period on-time (t_{on} —typically microseconds, where electrical discharge ignition leads to the formation of initiator radical species from the monomer) and then the longer period off-time (t_{off} —typically milliseconds, where conventional stepwise addition chain-growth monomer polymerisation proceeds) [12,13]. Excellent structural retention of the monomer functional groups is attained to yield well-defined functional polymer nanocoatings [12,14].

Chaetoceros calcitrans, *Chaetoceros mulleri*, and *Tisochrysis lutea* (T-Iso) are three predominant marine microalgae commonly grown in aquaculture as food for filter-feeding marine organisms, such as mussels and oysters, especially for the development of larvae in aquaculture hatcheries [15–17]. Under certain conditions, which are not fully understood, they exude extracellular polymeric substances which are slimy and stick to bioreactor walls, leading to biofilms and deterioration of quantity and quality of the algae production (likely by encouraging heterotrophic bacteria in the cultures) [18,19]. Reducing biofouling in the bioreactors for aquaculture hatcheries will likely lead to improved quality of the algal feed and lower maintenance of algal cultures (including alleviating the need for physical cleaning). Polyethylene film used to make bioreactor bags for continuous algae production was coated with a variety of functional nanocoatings prepared via pulsed plasmachemical deposition, Fig. 1. The structure-behaviour relationship of different wetting surfaces towards biofouling by marine microalgae was investigated and reported within.

2. Materials and methods

2.1. Pulsed plasma deposition

Photobioreactor polyethylene film (continuous algae production specification, 250 µm thickness, Amcor Flexibles NZ Ltd.) was used as the substrate material for biofouling studies. This was cleaned by sonication in a 50:50 vol mixture of propan-2-ol (+99.5 wt%, Fisher Scientific UK Ltd.) and cyclohexane (+99.7 wt%, Sigma-Aldrich Ltd.) for 15 min followed by air drying. Silicon wafer substrates were used for coating thickness measurements (orientation: <100>, resistivity: 5–20 Ω·cm, thickness: 525 ± 25 µm, front surface: polished, back surface: etched, Silicon Valley Microelectronics Inc.).

A cylindrical glass reactor (5.5 cm diameter, 475 cm³ volume) housed within a Faraday cage was used for plasmachemical deposition. This was connected to a 30 L min⁻¹ rotary pump (model E2M2, Edwards Vacuum Ltd.) via a liquid nitrogen cold trap (base pressure less than 2 × 10⁻³ mbar and air leak rate less than 6 × 10⁻⁹ mol s⁻¹) [20]. A copper coil wound around the reactor (4 mm diameter, 10 turns, located 10 cm downstream from the gas inlet) was connected to a 13.56 MHz radio frequency (RF) power supply via an L-C matching network. A pulse signal generator was used to trigger the RF power supply. Prior to film deposition, the whole apparatus was scrubbed using detergent and hot water, rinsed with propan-2-ol, oven dried at 423 K, and further cleaned

using a 50 W continuous wave air plasma at 0.2 mbar pressure for 30 min. Either photobioreactor polyethylene film or silicon wafer substrate was then inserted into the plasma reactor. For the case of silicon wafers, a 50 W continuous wave air plasma at 0.2 mbar pressure for 30 min was used for further cleaning prior to pulsed plasma nanocoating deposition. The monomer precursor was loaded into a sealable glass tube, degassed via several freeze-pump-thaw cycles, and then attached to the reactor. Monomer vapour was then allowed to purge the apparatus at a pressure of typically 0.15–0.20 mbar for 15 min prior to electrical discharge ignition. An initial continuous wave plasma was run for 30 s to ensure good adhesion to the substrate before switching to pulsed mode to deposit structurally well-defined functional nanocoatings over 30 min, Supplementary Material Table S1. Upon electrical discharge extinction, the precursor vapour was allowed to continue to pass through the system for a further 15 min, and then the chamber was evacuated to base pressure followed by venting to atmosphere and removal of substrates.

Monomers utilised for pulsed plasmachemical deposition were: 4-vinylpyridine (95%, Sigma-Aldrich Inc.), *N*-acryloylsarcosine methyl ester (+97%, Lancaster Synthesis Ltd.), glycidyl methacrylate (97%, Sigma-Aldrich Inc.), and 1H, 1H, 2H, 2H-perfluoroctyl acrylate (+95%, Fluorochem Ltd.).

For zinc oxide coatings, pulsed plasma poly(4-vinylpyridine) functionalized surfaces were immersed into an aqueous catalyst solution containing 2 µM palladium(II) chloride (+99.999%, Alfa Aesar, Fisher Scientific UK Ltd.), 3.0 M sodium chloride (+99.5%, Sigma-Aldrich Inc.), and 0.5 M sodium citrate dihydrate (+99%, Sigma-Aldrich Inc.) (which had been adjusted to pH 4.5 with citric acid monohydrate (+99%, Sigma-Aldrich Inc.)) for 12 h, and subsequently washed in deionized water [21]. Next, the palladium(II) chloride immobilized surfaces were placed into an aqueous chemical bath containing 0.05 M zinc nitrate (+98%, Sigma-Aldrich Inc.) and 0.05 M dimethylaminoborane (+97%, Sigma-Aldrich Inc.) at pH 6.5 and a temperature of 323 K for 2 h. Following zinc oxide growth, the surface was rinsed with deionized water.

2.2. Coating characterisation

Coating thicknesses were measured using a spectrophotometer (model nkd-6000, Aquila Instruments Ltd.). Transmittance-reflectance curves (350–1000 nm wavelength range) were acquired for each sample and fitted to a Cauchy model for dielectric materials using a modified Levenberg–Marquardt algorithm [22].

Sessile drop static contact angle measurements were carried out at 293 K using a video capture apparatus in combination with a motorised syringe (model VCA 2500XE, A.S.T. Products Inc.). Droplets of ultrapure water (2.0 µL) were employed. Measurements were repeated at least 3 times.

A Fourier transform infrared spectrometer equipped with a liquid nitrogen cooled MCT detector (model Spectrum One, PerkinElmer Inc.) was used to collect spectra across the 400–4000 cm⁻¹ range at 4 cm⁻¹ resolution and averaged over 265 scans. Attenuated total reflectance (ATR) infrared spectra of samples were acquired using a diamond ATR accessory (model Golden Gate, Graseby Specac Ltd.).

2.3. Immersed coating stability

Pulsed plasma poly(4-vinylpyridine) was coated onto silicon wafers. The coated silicon wafers were then fully immersed into deionized water (100 ml) at 293 K for predetermined periods of time and then removed for air drying at 293 K, followed by coating thickness measurement. Afterwards, the coated silicon wafers were re-submerged into the deionised water, and the process repeated for extended time intervals.

For the uncoated and pulsed plasma poly(4-vinylpyridine) coated polyethylene film samples used for *Chaetoceros calcitrans*, *Chaetoceros mulleri*, and *Tisochrysis lutea* (T-Iso) microalgae toxicity testing, these were analysed following removal from the microalgae solutions, and

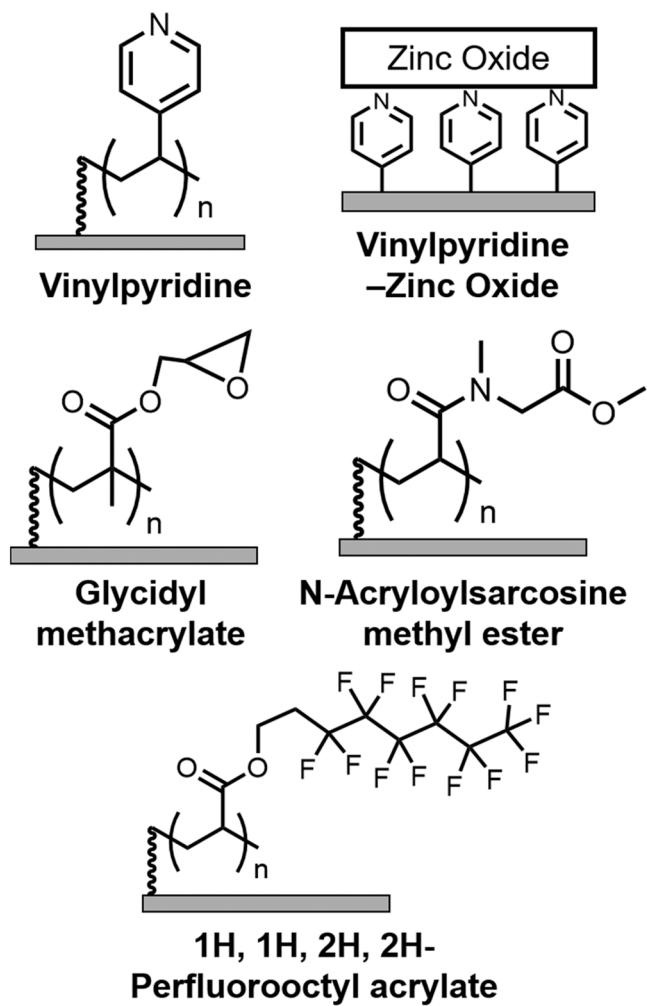


Fig. 1. Functional nanocoatings deposited onto solid substrate, showing pulsed plasma polymer repeat units and corresponding precursor names.

subsequently after immersing into cyclohexane (+99.5%, Fisher Scientific UK Ltd.) solvent followed by air drying prior to surface analysis.

2.4. Algae cultures and growth conditions

The algal species used in these experiments had been grown in a consistent environment for several months preceding experimental exposure. *Chaetoceros calcitrans* inoculum cultures were obtained from the Australian National Algae Culture Collection (ANACC) as CS178, and *Tisochrysis lutea* (T-Iso) and *Chaetoceros mulleri* from stocks originally obtained from the Scottish Association of Science (SAMS) Culture Collection of Algae and Protists (CCAP) as CCAP927/14 and CCAP1010/3 respectively. Algae stocks were maintained in 500 ml Erlenmeyer flasks containing 0.35 µm filtered seawater (FSW) that had been previously autoclaved at a salinity of 35–36 ppt using F/2 culture medium nutrient [23] (Varicon Aqua Ltd., product F2P). For *Chaetoceros calcitrans* and *Chaetoceros mulleri*, silica was added separately to the medium in the form of sodium metasilicate at a final concentration of 105.6 µM. Stock cultures and experimental cultures were kept at 294 K and irradiated with continuous white fluorescent light at ~100 µmol photons m⁻² s⁻¹.

2.5. Biofouling testing

Five different nanocoatings were assessed for their anti-biofilm performance in continuous cultures of the diatom *Chaetoceros calcitrans* in plastic bag-based photobioreactors as described previously [18]. Test samples of nanocoated polyethylene film were glued (superglue) to plastic frames that were made from food-grade container lids. Experiments indicated superglue was suitable for this work and did not have any effect on algae cultures when used in this way after drying. An untreated control substrate sample (250 µm thick photobioreactor polyethylene film, Amcor Flexibles NZ Ltd.) was glued next to each nanocoated sample using the same substrate material, Fig. 2(a). The test frames were inserted into bag tubing and heat-sealed to form short photobioreactor bags (circa 15 L volume, and these are referred to as Test Bags). Triplicate samples and control (15 Test Bags in total) were hung in 2 rows with random distribution of these samples, Fig. 2(b). *Chaetoceros calcitrans* microalgae was produced continuously in 18 longer photobioreactor bags (38 L volume, referred to as Production

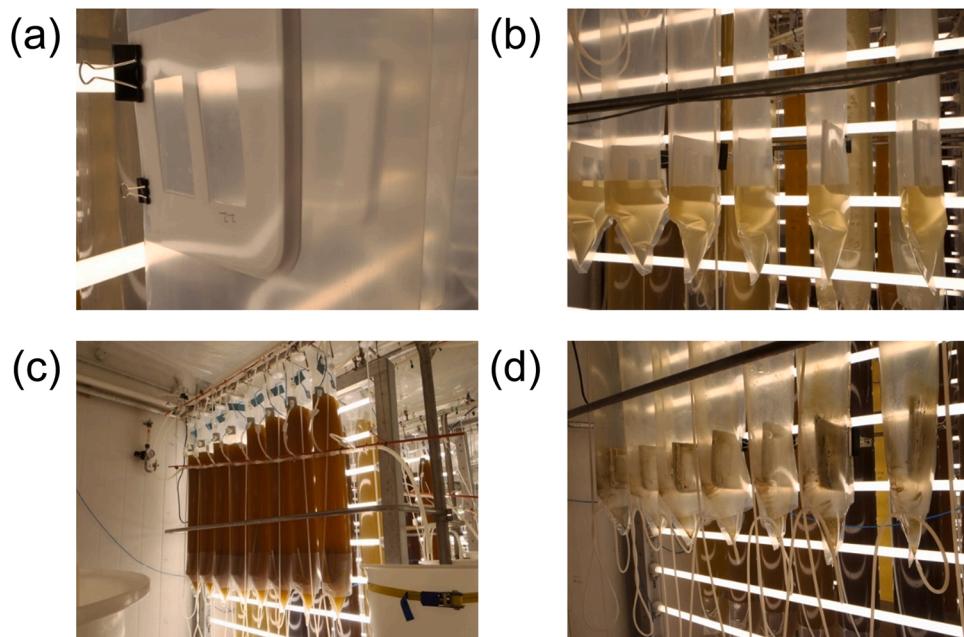


Fig. 2. (a) A sample frame inside a plastic photobioreactor bag clamped into position before filling with microalgae culture (the external black temporary positioning clips pushed off when the bags became rounded during filling with microalgae culture); (b) in the foreground are the lower volume Test Bag photobioreactors containing samples and partially filled with fresh microalgae culture (the larger photobioreactor Production Bags generate microalgae culture which feed into the Test Bag photobioreactor Production Bags can be seen in the background); (c) good uniform cultures in all bags (uniform colour) with the microalgae concentration visibly higher in all Test Bags compared to the algae being supplied from the Production Bags' harvest container, indicating algae growth and that there are no major toxicological effects from the samples; and (d) following drainage of photobioreactor bags showing fouling on the bag walls and sample holder frames.

Nanocoating	Control Coating	Biofouling Score	Contact Angle
Pulsed plasma poly(vinylpyridine)		-2	$38 \pm 5^\circ$
Pulsed plasma poly(<i>N</i> -acryloylsarcosine methyl ester)		-2	$52 \pm 3^\circ$
Pulsed plasma poly(glycidyl methacrylate)		-1	$56 \pm 2^\circ$
Zinc oxide		-1	$150 \pm 1^\circ$
Pulsed plasma poly(1H,1H,2H,2H-perfluoroctylacrylate)		+1	$122 \pm 3^\circ$

Fig. 3. Biofouling following 20 days exposure to *Chaetoceros calcitrans* microalgae in photobioreactor. The control untreated polyethylene film substrate mounted in the left window of each pair, and the coated polyethylene film mounted in the right window. The relative biofouling score was on the following scale: significantly cleaner than control (-2); marginally cleaner than control (-1); equal to control (0); marginally more fouled than control (+1); significantly more fouled than control (+2).

Bags). The harvest lines from each of these Production Bags were fed into a pooled harvest container. From this harvest container, the culture was pumped into the two rows of Test Bags containing the test samples and control samples. Each sample bag received about 27 L of fresh culture per day in 2.5 s pulses from a pneumatic pump. The bags overflowed into different harvest bins. The cultures were aerated. Production and Test Bags were cleaned occasionally with 1–3 day intervals (it was not deemed necessary to do this every day from experience with production cultures, and all the test samples received the same regime). This involved turning off the air, tapping the bags, letting flocs of algae settle to the bottom of the bags for 15 min, draining the accumulated flocs, and turning the air back on. The algae concentration in all Test

Bags was visibly higher than in the first set of harvest bins from Production Bag rows of bioreactors. This indicates that there was algae growth in all the Test Bags and that there was no major toxic effect from the test samples. After 20 days, the Test Bags were drained and biofouling on the Test samples observed visually macroscopically and scored for fouling using a qualitative scale (microscopic assessment was not valuable because of the patchiness of coverage). Care was taken to avoid contact between bag walls and samples so as not to remove any fouling present.

2.6. Toxicity testing

For microalgal toxicity evaluation, triplicate 300 ml batch cultures of *Chaetoceros calcitrans*, *Chaetoceros mulleri*, and *Tisochrysis lutea* (T-Iso) in 500 ml Erlenmeyer flasks were used to test the toxicity of the coated plastic films to algae. Cultures containing 0.35 µm filtered seawater that had been previously autoclaved at a salinity of 35–36 ppt using F culture medium nutrient (Varicon Aqua Ltd., product F2P) [23]—this was used at double strength so as to extend the life of the batch culture to provide a longer exposure time of the algae to the test samples (otherwise these cultures would run out of nutrients and die in about 4–5 days). Each of the four different batch coated films and a non-coated control of polyethylene film were fixed into a 35 mm photographic slide frame, by ‘clipping’ them into the frame (no glue was required), which had a 1 mm thick length of silicone tube fixed to it so it could be suspended in the culture, [Supplementary Material Fig. S1](#). Cultures were inoculated on day 0 with 40 ml of a parent stock and randomly placed on a shelf with a light frame. Experimental cultures were static but were swirled once every day during the testing period and moved to a new random location on the shelf after each sampling to control for varying distances from the light frame and therefore varying light intensities. The flasks were opened in a sterile laminar flow cabinet for sampling, where a 2 ml aliquot was removed using a sterile pipette. The experiment was carried out for 14 days with either 10 or 11 samplings occurring over that period.

For photosynthetic parameter analyses stress measurements (algae health), a Pulse Amplitude Modulation (PAM) fluorescence analyser (model Aquapen AP 110-C, Photon Systems Instruments spol. s r.o.) was used to measure the dark-adapted quantum yield (F_v/F_m) as an indicator of stress in the algae, reflecting photosynthetic performance of the algae [17]. Algae were diluted 50% in culture media for measurement and the dark-adaption period was 10 min. Optical density (OD) of the algae was also recorded with the same instrument using absorbance of light at 720 nm (OD720) as a proxy for cell number in the culture, and at 680 nm (OD680) as an indicator of light scattering and chlorophyll absorption, as well as fluorescence—all of which were used to compare algae growth rates for the different sample batches. The order of measurements on this instrument was fluorescence first, then quantum yield, then OD on the same sample, so that the measurements did not interfere with each other. The instrument was used with a 455 nm wavelength measuring light, and for F_v/F_m , a 30 µs pulse of 0.0135 µmol m⁻² was used for the flash pulse and 1650 µmol photons m⁻² s⁻¹ was used for the saturating pulse. The dilution, dark adaption period and intensity of both the flash and saturating pulses were optimised for these algae in separate experiments and the flash pulse was determined not to drive photosynthesis (it is not actinic) for these algae under these conditions. Statistical analysis was performed by repeated measure ANOVA of contiguous measurements (e.g. day 1 through day 4, or day 7 through day 9) and a mixed-effects analysis for the period day 0 through day 9, of F_v/F_m for each experimental film versus untreated control, using Prism 9 software for MacOS. Though experiments continued past day 9, statistical analysis was performed on data ahead of day 9 before any cultures reached stationary phase.

3. Results

3.1. Biofouling

Biofouling on the test samples were scored visually at the macroscopic level using a qualitative scale. Microscopic observations were not as valuable because of the patchiness of biofouling. The score scale was: significantly cleaner than control (−2); marginally cleaner than control (−1); equal to control (0); marginally more fouled than control (+1); and significantly more fouled than control (+2). The best antifouling performance for *Chaetoceros calcitrans* microalgal cultures was observed for pulsed plasma poly(4-vinylpyridine); whilst pulsed plasma poly

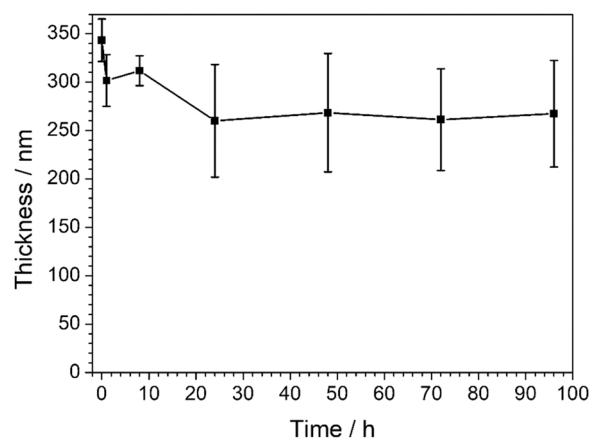


Fig. 4. Pulsed plasma poly(4-vinylpyridine) coating thickness variation with time of immersion in water. (Mean values, error bars represent standard deviation, n = 3).

(1 H, 1 H, 2 H, 2 H-perfluoroctylacrylate) gave rise to more biofouling compared to the control uncoated polyethylene substrate after 20 days, [Fig. 3](#). Pulsed plasma poly(glycidyl methacrylate), pulsed plasma poly(*N*-acryloylsarcosine methyl ester), and zinc oxide also displayed some level of biofouling hindrance, but were found not to be as consistent as pulsed plasma poly(4-vinylpyridine) coatings.

The best-performing pulsed plasma poly(4-vinylpyridine) coating was further examined for aqueous stability by measuring layer thickness on silicon wafers as a function of immersion time under water, [Fig. 4](#). The coatings showed a small decrease in thickness to eventually plateau after 24 h—this is likely due to some loosely bound low molecular weight polymer chains initially being washed off. No further loss of material after this time confirmed that the pulsed plasma poly(4-vinylpyridine) coating is stable in water.

Following microalgae exposure in flask batch experiments, the sample surfaces were analysed using infrared spectroscopy, [Fig. 5](#). The as-removed surfaces all displayed very similar infrared absorbance features, which can be attributed to surface settlement during removal from the microalgae solution. This settlement layer could be easily washed off using cyclohexane (as found for the control uncoated polyethylene film sample, requiring just 5 s immersion into the solvent). Even after 30 min immersion in cyclohexane, the pulsed plasma poly(4-vinylpyridine) coated polyethylene samples which had been exposed to microalgae in solution still exhibited infrared absorbance peaks characteristic of pulsed plasma poly(4-vinylpyridine) at around 3300 cm⁻¹ (O—H or N—H bonds from absorbed water [24,25]), 1650 cm⁻¹ (C=C stretching), and 1090 cm⁻¹ (aromatic C=C—H in-plane bends, and/or C—N stretches) [26–28]. Given the hydrophilic nature of poly(4-vinylpyridine), these absorbances are a combination of adsorbed water and the pulsed plasma poly(4-vinylpyridine) coating.

3.2. Toxicity

The best-performing pulsed plasma poly(4-vinylpyridine) coating was further examined for toxicity using a broad suite of algal health characteristics in flask batch cultures of *Chaetoceros calcitrans*, *Chaetoceros mulleri*, and *Tisochrysis lutea* (T-Iso). Optical density measurements showed that cell number in the culture increased linearly over time, with no notable difference observed between the uncoated controls and pulsed plasma poly(4-vinylpyridine) coated polyethylene film samples using repeated measure ANOVA statistical analysis, [Fig. 6](#). The drop-off observed after day 10 is expected due to nutrient deprivation in the batch culture by that stage. Similarly, no difference was seen for the fluorescence or the photosynthetic health parameter of quantum yield F_v/F_m [17]. This confirms that there is an absence of toxicity for the

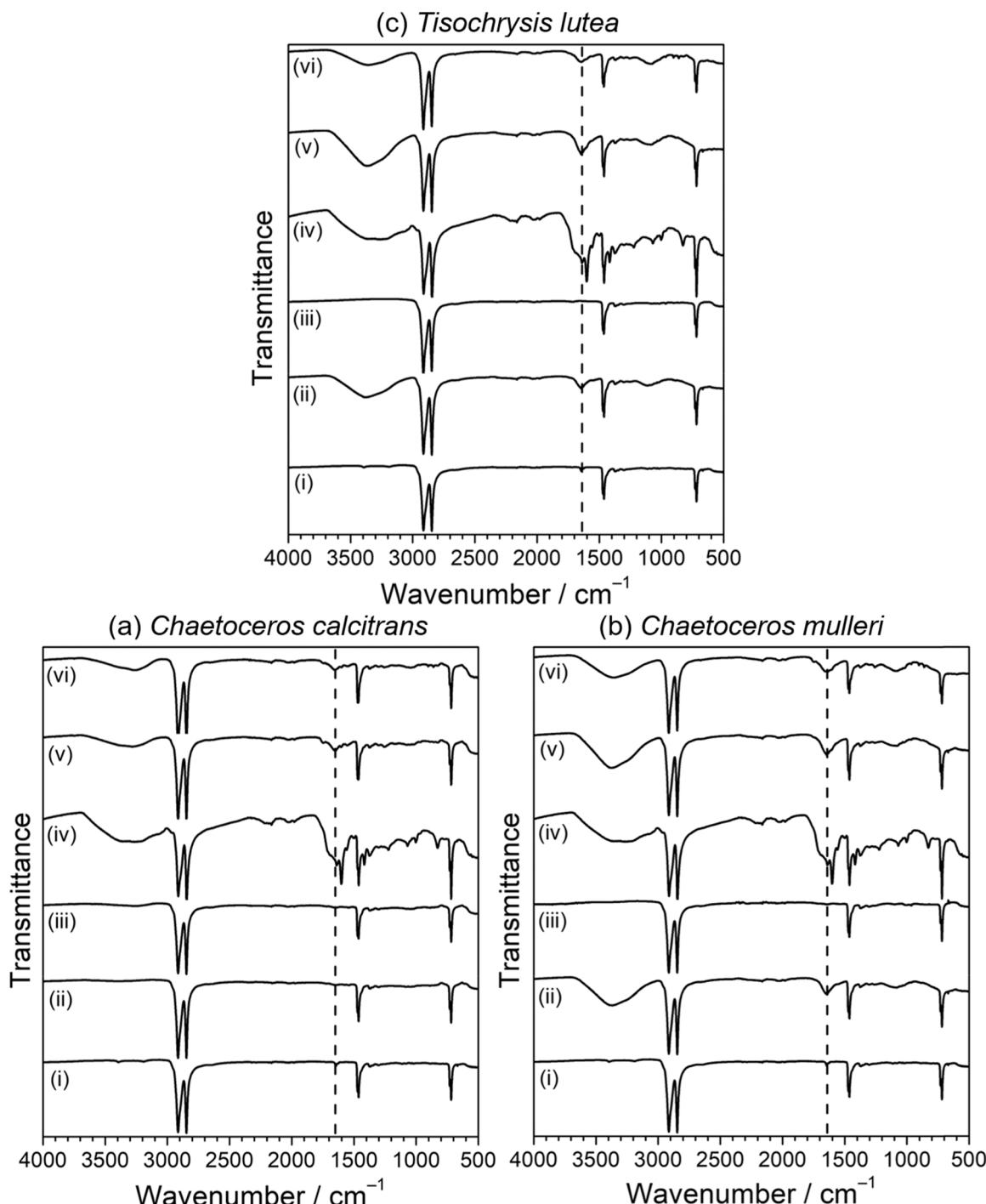


Fig. 5. ATR-infrared spectra of samples following immersion in batch flask microalgae cultures of (a) *Chaetoceros calcitrans*; (b) *Chaetoceros mulleri*; and (c) *Tisochrysis lutea*. Where: (i) control uncoated polyethylene (not exposed to microalgae); (ii) control uncoated polyethylene exposed to microalgae (washed with water afterwards only in the case of *Chaetoceros calcitrans*); (iii) following washing of (ii) with cyclohexane for 5 s; (iv) control pulsed plasma poly(4-vinylpyridine) coated polyethylene (not exposed to microalgae); (v) pulsed plasma poly(4-vinylpyridine) coated polyethylene exposed to microalgae (washed with water afterwards only in the case of *Chaetoceros calcitrans*); and (vi) following washing of (v) with cyclohexane for 30 min.

pulsed plasma poly(4-vinylpyridine) coatings towards the microalgae.

4. Discussion

The plasma deposited coatings investigated in this study do not utilise any antimicrobial additives and are ultrathin, which is important for addressing environmental toxicity and sustainability concerns. For the electroless deposited zinc oxide layer, the different crystal growth

planes provide sufficient surface roughness to yield inherent hydrophobicity as a consequence of the Cassie–Baxter effect trapped gas layer [21,29]. However, this air layer will collapse during prolonged immersion in aqueous media (within 24 h), leading to hydrophilicity due to contact of water with zinc oxide surface hydroxyl groups, which in conjunction with the antimicrobial properties of zinc oxide, provides some degree of antifouling performance [21]. Given that low duty cycle pulsed plasma polymer coating surfaces tend to be smooth, the water

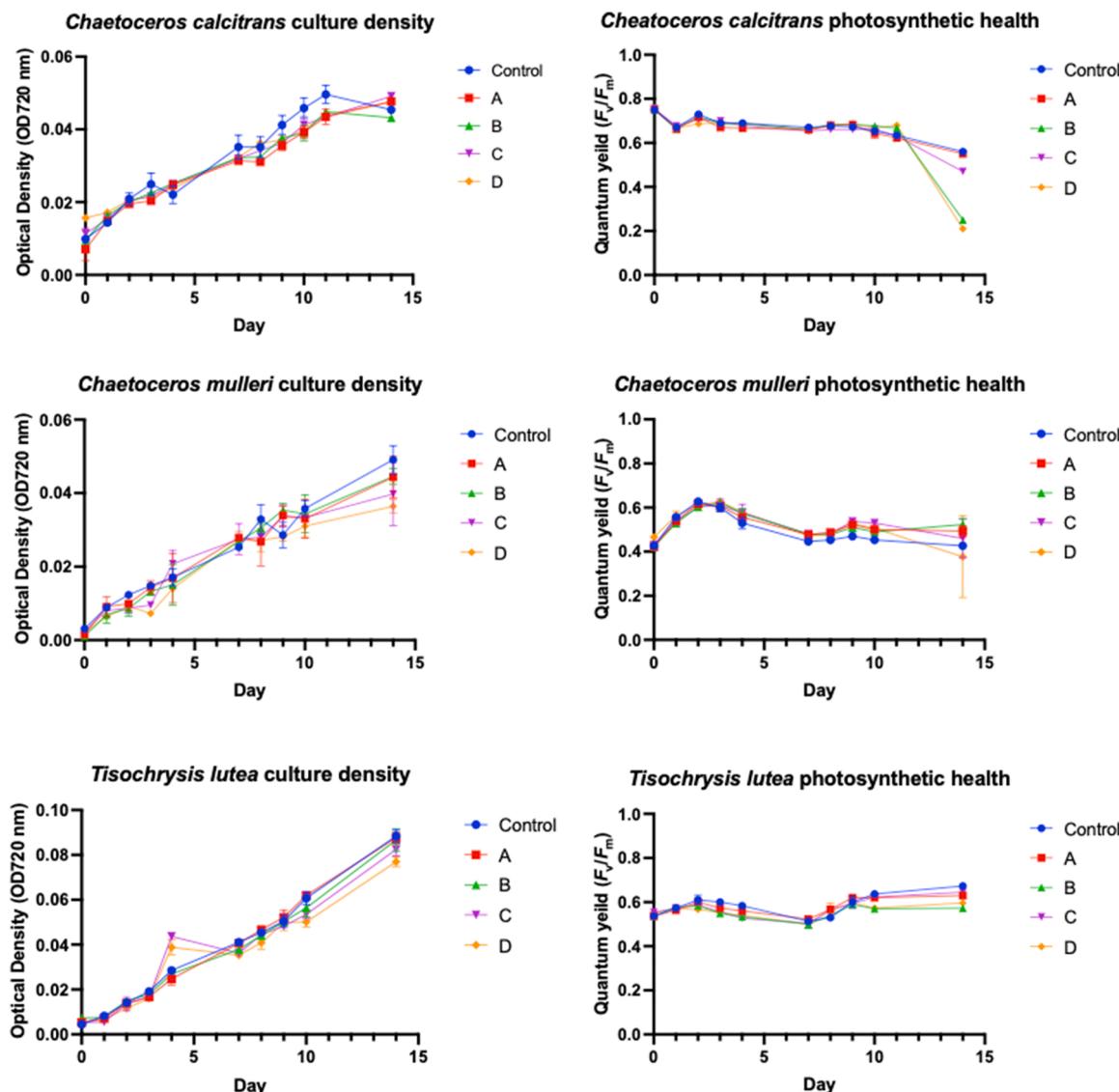


Fig. 6. Algae growth and photosynthetic health using optical density (720 nm) and quantum yield respectively of *Chaetoceros calcitrans*, *Chaetoceros mulleri*, and *Tisochrysis lutea* (T-Iso) microalgae versus time for uncoated (Control) and pulsed plasma poly(4-vinylpyridine) coated polyethylene film samples (A, B, C, D).

repellency of the pulsed plasma polymer layers correlates to the chemical functional groups [30,31]. The most hydrophilic nanocoating surface (pulsed plasma poly(4-vinylpyridine)) showed the best antifouling behaviour, Fig. 3. This correlation between water contact angle value and extent of biofouling (for non-Cassie–Baxter surfaces) is consistent with previous biofouling studies [32,33]. The good performance noted for pulsed plasma poly(*N*-acryloylsarcosine methyl ester) ties in with its protein resistance properties [34]. Hydrophilic surfaces are able to resist fouling due to the formation of a tightly-bound surface water layer which creates a barrier towards biofoulant attachment [35–37]. Such hydrophilic surfaces typically have the following four characteristics: (i) polar, (ii) hydrogen bond acceptor, (iii) no hydrogen bond donor groups, and (iv) charge neutral [37,38]. The pyridine molecule is known to form hydrogen bonds with water molecules (i.e. act as a hydrogen bond acceptor) and form a hydration sphere [39–42]. Hence, grafted poly(4-vinylpyridine) coatings are able to resist fouling by bovine serum albumin and human fibrinogen proteins, which is attributable to the presence of the hydration layer [27].

The physical structure of coatings can also play an important role in preventing biofouling of microalgae—for example, biomimetic silicon modified acrylic resin surface moulded from shark skin has been shown

to display good anti-biofouling against a range of microalgae due to its V-groove ripples and imbricate boundary structure preventing adhesion [43]. Hence, pulsed plasma poly(4-vinylpyridine) nanocoatings applied to such physical structures may enhance the antifouling performance further.

Prevention of biofouling using the nanocoatings described here may allow for the use of other materials in the construction of algal bioreactors. These materials could be more suitable for outside use and large-scale algal production or have programmed obsolescence incorporated with reduced environmental impact compared with polyethylene. These could include bio-based plastics that can be composted after use, but perform as well or better than current state-of-the-art materials with the addition of anti-fouling coatings.

While the prevention of fouling is important for many applications, the encouragement of fouling may be equally desirable, e.g. in bioreactors using surface-dwelling (benthic) algae or other microorganisms such as in immobilised fermentation, or in the collection of biomass from dilute suspensions where the usual physical methods (filtration, centrifugation etc.) are not feasible or expensive (e.g. open ocean) [44,45]. And so, the utilisation of hydrophobic pulsed plasma nanocoatings (for example, hydrocarbon alkyl group variants) would be

well suited for such applications.

5. Conclusions

A structure-behaviour investigation has found that hydrophobic pulsed plasma poly(1 H, 1 H, 2 H, 2 H-perfluoroctylacrylate) coating experiences a high level of biofouling during exposure to marine microalgal species. In contrast, hydrophilic surfaces reduce the extent of biofouling, with the most hydrophilic coating, pulsed plasma poly(4-vinylpyridine) performing the best. Furthermore, this coating was found to be non-toxic towards marine microalgae species *Chaetoceros calcitrans*, *Chaetoceros mulleri*, and *Tisochrysis lutea* (T-Iso), which are used widely in aquaculture settings.

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CRediT authorship contribution statement

J. P. S. B., H. F. K., and M. A. P. devised the concept. Sample preparation, water contact angle, and infrared spectroscopy were performed by H.J.C. Microalgae biofouling tests was carried out by H. F. K., I. C., and M. A. P. The manuscript was jointly drafted by J. P. S. B., H. J. C., and M. A. P. All authors gave final approval for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material

Photographs of microalgal toxicity experiments and pulsed plasma deposition parameters are supplied as Supplementary Material.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.colsurfa.2022.128313.

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