Synthesis, Characterization, and Evaluation of Antifouling Polymers of 4-Acryloyloxybenzaldehyde with Methyl Methacrylate

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Received 23 May 2006; accepted 2 February 2008
DOI 10.1002/app.29313
Published online 24 February 2009 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: In recent years, antifouling (AF) polymers are widely used in marine paints to protect the ship hulls from biofouling. The AF polymer coatings have better leaching characteristics and long lasting efficiency than other conventional formulations. In this study, an attempt has been made to prepare new *p*-acryloyloxybenzaldehyde(AcBA) polymers to assess their AF efficiency against marine microfoulers. The monomer, AcBA was prepared by the esterification reaction between *p*-hydroxybenzaldehyde (HBA) and acryloyl chloride (Ac) in presence of triethylamine (TEA) in MEK at 0°C. The reaction was monitored by TLC and the prepared monomer was characterized by UV, IR, ^{1}H-NMR and GC-MS. The homo-[poly(AcBA)] and co-polymers [poly(AcBA-*co*-MMA)] were prepared by solution polymerization using BPO as initiator. To find out the AF activity of prepared polymers, representatives of marine microfoulers, shipfouling bacteria (*Bacillus macroides* and *Pseudomonas aeruginosa*) and microalgae (*Amphora coffeaeformis* and *Navicula incerta*) were screened. The contact toxicity and diatom attachment assays were conducted with prepared polymers and microfouling formation on coatings was also investigated using a tubular biofilm reactor. AF potential of these polymers coating is demonstrated. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 112: 2741–2749, 2009

Key words: AcBA; addition polymerization; antifouling polymers; copolymerization; microfouling

INTRODUCTION

Biofouling on ship hulls causes severe damage to the shipping industry. To protect the ship hull and prevent the attachment of fouling organisms, AF coatings are being used. In recent years, AF polymers are prominently used in the shipping industries. The AF polymers have many advantages over conventional AF formulations. The controlled leaching rate of AF polymers may decrease the risk of environmental pollution, whereas the conventional soluble or insoluble matrix types of AF coatings are largely responsible for the environmental pollution.

Antimicrobial polymers are widely used in various industrial applications. In recent years, it is also employed in AF coatings to prepare effective AF polymer formulations. The use of antimicrobial polymers offers a promise for enhancing the efficacy of some existing antimicrobial agents and minimizing the environmental problems accompanying conventional antimicrobial agents by reducing the residual toxicity of the agents, increasing their efficiency and selectivity and prolonging the lifetime of the antimicrobial agents.[1] The polymeric antimicrobial agents also have the advantage that they are nonvolatile, chemically stable and do not permeate through the skin. Therefore, they can reduce losses associated with volatilization, photolytic decomposition and transportation.[2] In a previous study, the antimicrobial activity of the quaternary ammonium and phosphonium copolymers was reported.[3] Similarly, monomer and polymers of benzoic acid derivatives were screened as antimicrobial agents.[4] The 2,4,4′-trichloro-2′-acryloyloxydiphenylether and its homo- and copolymers with methyl methacrylate and styrene, respectively, were synthesized[5] and then, their biocidal activity was tested against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Acrylic polymers containing pentachlorophenol was reported as potential biocides for coating formulations.[6–8] The fungicidal activities of the *N*-acryloyl-2-(4′-thiazolyl) benzimidazole (AcTBZ), poly(AcTBZ), and poly (AcTBZ-*co*-AA) were investigated against *Aspergillus niger* and *Chaetomium globusum*.[9] The controlled release rate of the organotin monomer and their polymers containing *p*-acryloyloxybenzoic acid was reported.[10] Benzaldehyde is an environmentally safe chemical and its substituted forms are widely used for various applications such as bactericide,[11–14] fungicide,[15–17] algaecide[18] and predominantly used in biotransformation process to prepare l-phenyl acetyl carbinol, a precursor for ephedrine (a value added

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Contract grant sponsors: Ministry of Land, Transport, Maritime Affairs and Fisheries, South Korea.

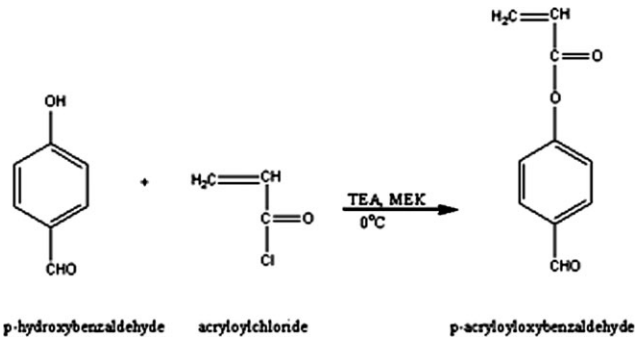

Figure 1 Synthesis of 4-acryloyloxybenzaldehyde (AcBA).

product)[19] and also used extensively as intermediates to synthesize medicines.

Considering its broad spectrum inhibitory activities against bacteria, fungi, and algae, in the present investigation *p*-acryloyloxybenzaldehyde (AcBA) was synthesized and employed to prepare acrylic copolymers which may be useful in AF paints, coatings, and adhesives. This article discusses the synthesis and characterization of AcBA monomer and its polymers. The homo- and copolymers have been characterized for their efficiency against microfouling organisms such as marine bacteria (*Bacillus macroides* and *Pseudomonas aeruginosa*) and diatoms (*Amphora coffeaeformis* and *Navicula incerta*).

EXPERIMENTAL

Materials

Acryloylchloride (Ac) (96%), *p*-hydroxybenzaldehyde (HBA) (99%), Triethylamine (TEA) (99.5%) were procured from Acros Chemical, Belgium. The benzoyl peroxide (BPO) was purchased from Sigma Aldrich. Methyl methacrylate (MMA) (99%), methyl ethyl ketone (MEK) (98%), *n*-hexane, toluene, methanol were procured from Samchun Chemicals (South Korea). All the chemicals were used without any further purification.

Synthesis of *p*-acryloyloxybenzaldehyde (AcBA)

The procedure of AcBA synthesis was illustrated in Figure 1. Briefly, 10 g (0.08 m) of HBA and 10 g (0.1 m) of TEA were dissolved in 100 mL of MEK in a 250 mL four neck round-bottomed (RB) flask. The RB flask was equipped with stirrer and thermometer. And then, 9 g (0.1 m) of Ac in 30 mL of MEK was added slowly in to the RB flask at 0°C by using a dropping-funnel. After the addition of Ac, the reaction mixture was stirred for 3 h at 0°C and 3 h at room temperature. The reaction mixture was filtered to remove triethylamine hydrochloride and then, the filtrate was successfully washed by 5% $NaHCO_3$ solution and with distilled water. The Na_2SO_4 was added into the filtrate and kept over night to remove the traces of water. And then, the filtrate was concentrated by rotoevaporation and the AcBA was obtained by dropping the concentrated filtrate into an excess of *n*-hexane. Finally, the precipitated AcBA (pale yellow flakes) was dried at room temperature under reduced pressure for 24 h. The reaction was monitored by TLC and the products were characterized by GC-MS, IR, and NMR (Fig. 2). The melting point of the AcBA was 103–107°C. The purity of *p*-acryloyloxybenzaldehyde (AcBA) was 96.5% determined by HPLC.

Polymerizations

The homo- and co-polymers of AcBA were prepared by the solution polymerization with MMA as shown in Figure 3. The required quantities of AcBA, MMA, toluene, and BPO were mixed in polymer tube equipped with magnetic stirrer and a septa cap. The solution was deoxygenated by purging with purified N_2 gas. The tube was sealed and placed in a regulated thermostat bath at 70°C for fixed periods of time. The polymer solution obtained was precipitated in excess methanol. The precipitate was collected by filtration and dried at room temperature under vacuum. The copolymer *p*-acryloyloxybenzaldehyde-*co*-methyl methacrylate [poly(AcBA-*co*-MMA)] was prepared by the aforementioned procedure by using monomers, AcBA and MMA in the feed ratio of 4 : 6 (mole ratio).

Characterization

The infrared spectroscopy IR [Bruker, Germany), IFS88/Perkin Elmer, Spectrum GX on solid samples in KBr pellets] was used to investigate the presence of the ester carbonyl group in the AcBA. ^{1}H-NMR spectra were obtained on a JNM-AL400 spectrometer

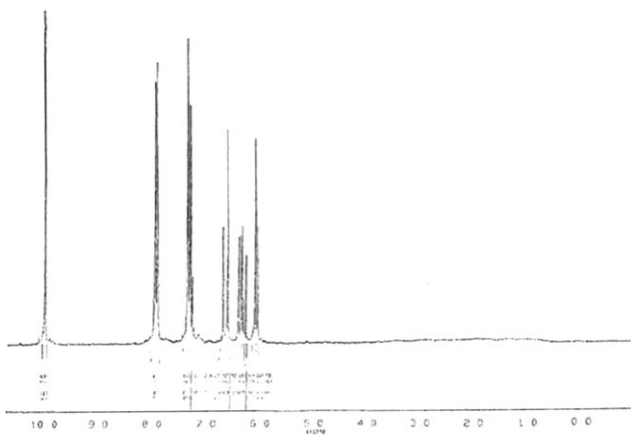

Figure 2 The ^{1}H-NMR spectrum of *p*-acryloyloxybenzaldehyde (AcBA).

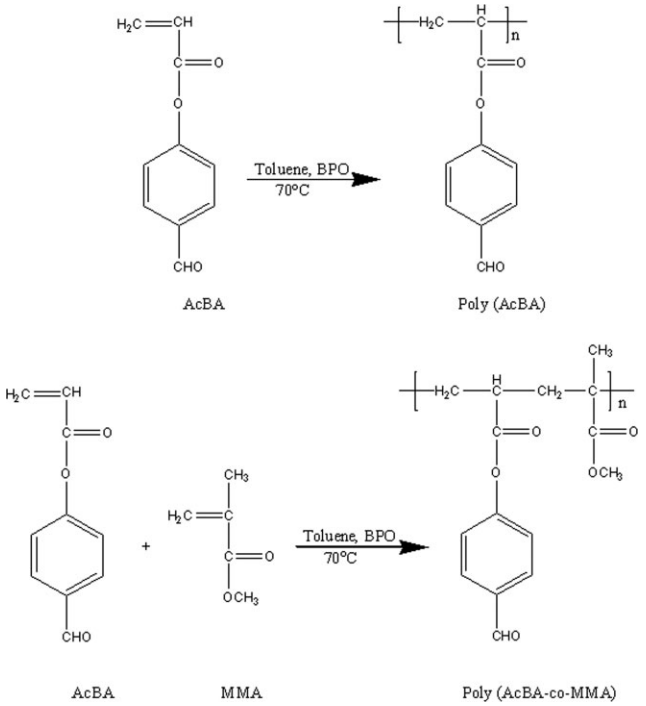

Figure 3 Synthesis of poly(AcBA) and poly(AcBA-*co*-MMA).

(Jeol, Akishima, Japan) at 400 MHz, respectively. The number and weight average molecular weights and molecular weight distributions were determined using a gel permeation chromatography (GPC) equipped with an Agilent 1100 series RI detector, quaternary pump, and two PLgel 5-μm MIXED-D and E columns in set, and calculated with poly(ethylene glycol) standards. The flow rate was 1 mL/min and the temperature (both the column compartment and the flow cell of the refractive index detector) was kept at 35 ± 0.1°C. The mass of the AcBA was determined by GC-Mass spectroscopy.

Microfouling assays

Antibacterial activity

Antibacterial assay was conducted with two marine bacteria, *Bacillus macroides* (strain KORDI-13724) and *Pseudomonas aeruginosa* (strain KORDI-13716). Assays were conducted using paper disc method. To prepare 0.5–3.0 mg/disc concentrations, aliquots of homo- and co-polymers; and PMMA were dissolved in THF and then loaded to paper discs (6 mm; Advantec, Japan); and dried at room temperature for about 12 h. Bacterial cultures were raised in marine broth-2216 (DIFCO). At log phase of growth, it was inoculated onto agar plate prepared with marine agar-2216 (DIFCO) and spread uniformly to make bacterial lawn. Test polymer-loaded paper discs were placed on the bacterial lawn and agar plates were incubated for 48 h at 28°C. Tetrahydrofuran (THF) and triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether) were used as negative and positive controls, respectively. At the end of the experiments, inhibition zone formed around the disc (x) was calculated by subtracting the disc size (6 mm) from the zone diameter and then dividing by two. Three replicates were used and results expressed as mean ± SD.

Contact toxicity

The effect of test polymers on the growth of *Dunaliella tertiolecta* Butcher (KMCC C-010) was tested in the direct contact test. The bottom of glass tubes (15 × 150 mm^2) were coated with PMMA, prepared homo- and co-polymers. To prepare 0.063–2.0 mg cm^{-2} of coatings, test substances were dissolved in 0.5-mL THF and spread on the bottom of each glass tube. Coatings were allowed to dry at room temperature for 6 h. Later, 4 mL of microalgal culture medium[20] was added to each tube and inoculated with 100 μL (1×10^4 cells) of *D. tertiolecta* culture in the exponential growth phase. Culture tubes were incubated in growth chamber at 20°C under 50 μE m^{-2} s^{-1} with 12 : 12 LD cycle. Growth of *D. tertiolecta* was monitored after 96 h exposure time. Results are mean of three individual replicates (±SD).

Diatom attachment

The shipfouling microalgae, *Amphora coffeaeformis* (KMCC B027) and *Navicula incerta* (KMCC B001) cultures were raised in microalgal culture medium. Culture flasks (1 L) were kept in a growth chamber at 20°C under 50 μE m^{-2} s^{-1} with 12 : 12 LD cycle. At exponential growth phase cultures were repeatedly centrifuged (3000 rpm from 5 min) and the supernatants were discarded. Later, *A. coffeaeformis* cells were washed with filtered (0.45 μm) sterile seawater. Finally, cell pellet was redissolved in filtered (0.45 μm) sterile seawater and passed through double fold of nylon mesh to get cell suspension with single cells. The initial cell density of diatom cell suspension was counted using haemocytometer and necessary dilutions were made to obtain 1×10^5 cells mL^{-1}.

Five milliliters of cell suspensions of *A. coffeaeformis* or *N. incerta* were used for diatom attachment assays. Experiments were conducted in glass petridishes (60 × 15 mm^2) with 15 mL of filtered (0.45 μm) sterile seawater and diatom cell suspension (5 mL). Surface of the glass petridishes were previously coated (2 cm^2) with different concentrations of test polymers. Petridishes were kept in growth chamber under fluorescent light (50 μE m^{-2} s^{-1}) for 12 h. Later, cell suspension was discarded and loosely

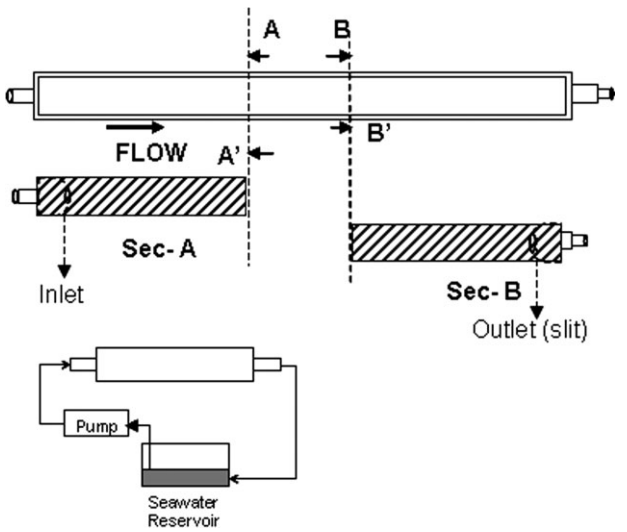

Figure 4 A cross-sectional view of tubular biofilm reactor (A: inlet and B: outlet). Lower inset showing total biofilm reactor with seawater circulation system.

attached cells were washed with 5 mL of sterile seawater and attached diatom cells were counted (10–20 fields: 400×) under light microscope (Olympus CK 2, Japan). Mean diatom cell attachment was calculated from three replicates and results were expressed as mean (±SD) with level of significance (*t*-test) $P < 0.05$; P (0.01).

Biofilm formation

Standard microscopic glass slides (75 × 25 × 1 mm^3) were used for the biofilm formation experiments. Slides were soaked overnight in 10% HCl and repeatedly washed with distilled water and then kept in hot air oven (100°C) for 6 h. Acid-cleaned slides were coated (2 cm^2) with different concentrations (0.5–3 mg) of test polymers. To prepare stock solutions, test polymers were separately dissolved in 10 mL of THF. Coated slides were dried at room temperature.

Biofilm formation of diatoms on test polymer surface was investigated using a tubular biofilm reactor (Figure 4). The design of the reactor is based on criteria of previous studies.[21,22] Table I shows the characteristics of the tubular biofilm reactor. The setup was made with a horizontal half cylinder shaped long PVC duct (with 100-cm length and 15-cm dia.) in which seawater re-circulated and the top was covered with perspex plate. There was an inlet with check valve at the left end (A), whereas a long slit at the other end which serve as out let (B). A motorized pump (20 W) was equipped to lift the seawater from the reservoir (10 L) to the tubular biofilm reactor. The out let was directly connected to the reservoir which was kept 50 cm below the biofilm reactor. Artificial seawater (Instant Ocean, Aquarium Systems) was amended with microalgal culture nutrients and then used in the biofilm reactor. The volume of the seawater maintained in the PVC duct was 3.5 L and the flow rate was fixed at 504 L h^{-1}. The total setup was fixed under the fluorescent lamps to receive 50 μE m^{-2} s^{-1} of irradiance with 12 : 12 LD cycle. The biofilm reactor was operated at room temperature (22 ± 2°C) and seawater temperature was maintained at 20 ± 0.5°C using a standard aquarium heater (100 W) fixed in the reservoir. The pH of the circulating seawater was maintained at 8.2 ± 0.2. Slides with different types of coatings were randomly placed on the bottom of the tubular biofilm reactor. Before 12 h from the starting of each experiment cell suspension of *A. coffeaeformis* or *N. incerta* was added to the reservoir (1 × 10^4 cells mL^{-1}). After 48 h exposure, slides were carefully taken out with forceps and put into 100-mL glass beakers containing sterilized seawater (0.45 μm). Using paper towels diatom cells attached on the back side of each slide was wiped out and the biofilm formed on the front side polymer coating was examined under microscope as mentioned in the previous

TABLE I
Characteristics of Tubular Biofilm Reactor System

Parameters	
Temperature (°C)	20 ± 0.5
pH	8.2 ± 0.2
Salinity (psu)	30
Light source and L/D regime	White fluorescent; 12/12
Light Intensity (μE m^{-2} s^{-1})	50
Reactor dimension (L × W × H) in mm	1000 × 150 × 70
Reactor capacity and working volume (L)	5 and 3.5
Seawater reservoir (L)	10
Magnetic pump (Pan World NH-3PX) 60 Hz	20 W
Flow rate (L h^{-1})	504
Recirculation rate (L h^{-1})	144
Composition of artificial seawater	Instant Ocean with microalgal nutrients[20]

TABLE II
Antibacterial Activity of Test Polymers Against Two Marine Bacteria (Mean Inhibition Zone in mm ± SD)

	Bacillus macroides				*Pseudomonas aeruginosa*			
Conc. (mg/disc)	PMMA	BA	HP	CP	PMMA	BA	HP	CP
0.5	4.5 ± 0.5	–	2 ± 0.5	2 ± 0.8	3.5 ± 0.8	–	–	1 ± 0.5
1.0	6.5 ± 1.5	1.5 ± 0.5	4.5 ± 0.5	4.5 ± 0.8	5 ± 1	–	2.5 ± 0.5	1.5 ± 0.5
2.0	8 ± 1	2.5 ± 0.5	7.5 ± 1.3	6.5 ± 1.3	6 ± 1	2 ± 0.5	6.5 ± 0.5	4 ± 1.3
3.0	9 ± 0.8	4.5 ± 0.9	10.5 ± 1.3	9 ± 1	7 ± 1.3	3.5 ± 0.5	7.6 ± 0.5	6 ± 1.3
One-way ANOVA	**	**	**	**	*	**	**	**

There was no inhibition zone observed for untreated control discs. The mean inhibition zone observed for the solvent tetrahydrofuran (THF) was ≤1 mm. At 100 μg/disc, a positive control triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether) inhibited the growth of *B.macroides* and *P. aeruginosa* with 12.5 ± 0.9 mm and 10 ± 1.3 mm of inhibition zones, respectively.

PMMA, poly methyl methacrylate; BA, 4-hydroxybenzaldehyde; HP, poly(ACBA); CP, Poly(ACBA-*co*-MMA).

Asterisks indicate means of inhibition zones obtained for different concentrations of test compound are significantly different (one-way ANOVA $^{**}P < 0.01$; $^{*}P < 0.05$).

section. Later, biofilm from each test slide was carefully removed with a help of a fine brush and collected into glass tubes. The remaining diatom cells on each slides and brush were carefully washed using 1 mL of distilled water and transferred to glass tube. The total carbohydrate content of biofilm was determined using phenol-sulfuric acid method using glucose as standard.[23]

Data analysis

Results were tested by one-way analysis of varience (ANOVA), followed by Dunnett's post hoc comparisons (one-tailed/two-tailed) with the corresponding controls (surface without polymer coating) with each concentration of poly(AcBA) and poly(AcBA-*co*-MMA). Significant differences were considered at $^{**}P < 0.01$;$^{*}P < 0.05$ levels.

RESULTS AND DISCUSSION

Identification of *p*-acryloyloxybenzaldehyde (AcBA)

The synthesized AcBA was identified from its UV, IR, GC-MS, and ^{1}H-NMR (Figure 2) spectra. The IR spectrum shows characteristic absorption bands at 1598, 980 cm^{-1} (vinyl) and 1747 and 1696 cm^{-1} (C=O). The ^{1}H-NMR spectrum of AcBA (solvent, $CDCl_3$) exhibited several peaks at 5.80–6.17 (—CH), 6.00–6.68 (—CH_2), 7.17–8.00 (—Ar) and 9.97(—CHO) ppm. From the UV spectrum, the wavelength at maximum absorption was 263.5 nm. The mass spectrum exhibited the mass (m/e) was 176 (M^+).

Identification of homopolymers

The poly(AcBA) was identified from its IR spectrum indicating absorptions at 2900 cm^{-1}, characteristics of the vinyl polymer backbone, with disappearance of vinyl absorptions of monomeric AcBA at 1598, 980 cm^{-1}. The ^{1}H-NMR spectrum of poly(AcBA) (solvent, $CDCl_3$) exhibited several peaks at 1.20–1.90 (—CH_2), 2.2–2.50 (—CH), 7.1–7.3 (—Ar), and 9.90(—CHO). The number and weight average molecular weights of poly(AcBA) were 84,400 and 142,300, respectively. The poly(MMA) was identified by IR and ^{1}H-NMR spectra. The number and weight average molecular weights of poly(MMA) were 82,500 and 163,600, respectively.

Identification of copolymer

The IR spectrum of poly(AcBA-*co*-MMA) indicated the absorptions at 2900 (phenyl ring, AcBA), 1745 cm^{-1} (C=O, AcBA), 1729 cm^{-1} (C=O, MMA) with the disappearance of vinyl absorptions at 1598, 980 cm^{-1}. The ^{1}H-NMR spectrum poly(AcBA-*co*-MMA) (solvent, $CDCl_3$) was also identified by several peaks at 0.6–1.4 (—CH_3), 1.41–2.50 (—CH_2), 2.50–2.80 (—CH), 3.03–3.90 (—OCH_3), 7.17–8.00 (—Ar) and 9.90 ppm (—CHO). The number and weight average molecular weights of poly(AcBA-*co*-MMA) were 50,900 and 98,500, respectively and the polydispersity was 1.9.

Microfouling assays

The antibacterial activity of HBA, AcBA, homo- and co-polymers against two marine bacteria (*B. macroides* and *P. aeruginosa*) is given in Table II. *B. macroides* was found to be susceptible to all the test polymers (ANOVA, $P < 0.01$). A high inhibition zone of 10.5 ± 1.3 mm was observed against poly (AcBA) [3 mg/disc]. Bacterial colonies of *P. aeruginosa* were comparatively less inhibited. A homo polymer, poly(AcBA) effectively inhibited the growth of both bacteria at 3 mg levels. It was observed that the antibacterial activity of homo polymer was slightly higher than the copolymer.

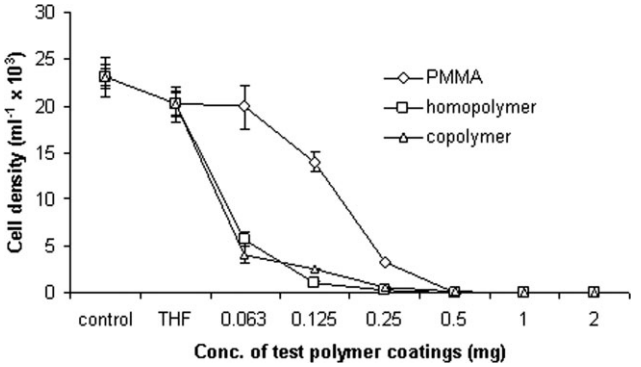

Figure 5 Contact toxicity of AcBA polymer coatings and PMMA (cm^{-2}) on *Dunaliella tertiolecta*. Exposed for 96 h.

In the contact toxicity tests, prepared polymer coatings effectively inhibited the growth of *D. tertiolecta* (Figure 5). At low concentration of 0.063 mg cm^{-2}, on coatings of homo- and co-polymers of AcBA, the growth of *D. tertiolecta* was fivefold decreased when compared to untreated control. PMMA was less toxic to *D. tertiolecta* cells.

A dose-dependent trend was seen in the attachment of microfouling diatoms on polymer coatings. On coatings made with 3 mg cm^{-2} of poly(AcBA-*co*-MMA), the attachments of *A. coffeaeformis* were significantly inhibited (Dunnett's test $P < 0.001$) (Figure 6). A homo polymer, PMMA coating was also exhibited significant inhibitory effects on the attachment of *A. coffeaeformis* (Dunnett's test P (0.001). The attachment of *N. incerta* was more on all the polymer test coatings when compared to *A. coffeaeformis* as shown in the Figure 7. On both homo- and co-polymers at 1 mg cm^{-2} significant differences in the *N. incerta* attachment were observed when compared to acid-glass control surfaces ANOVA, $P < 0.01$). Unlike *A. coffeaeformis*, *N. incerta* cells (10–20%) were able to attach on high concentration (3 mg cm^{-2}) of AcBA polymer surfaces.

Biofilm formation of *A. coffeaeformis* and *N. incerta* on the polymer coatings are shown in Figures 8 and 9. The attachment of both diatoms on all the test coatings was comparatively decreased over control. In contrast, when compared to *A. coffeaeformis* fairly high cell densities of *N. incerta* were estimated from test surfaces. The attachment of *N. incerta* on the coatings of both the homo- and copolymers was 66–83% decreased when compared to control [Figure 9 (ANOVA, $P < 0.01$)]. On 3 mg cm^{-2} coatings of both homo- and copolymer coatings, 70% of *A. coffeaeformis* attachment was inhibited (Dunnett's test, $P <$ 0.01). On the other hand, relatively high amount of extracellular polysaccharides (EPS) were found in biofilms of *A. coffeaeformis*. To estimate the biomass of biofilms (cm^{-2}), the total carbohydrate contents of biofilms were determined. In relation to high EPS

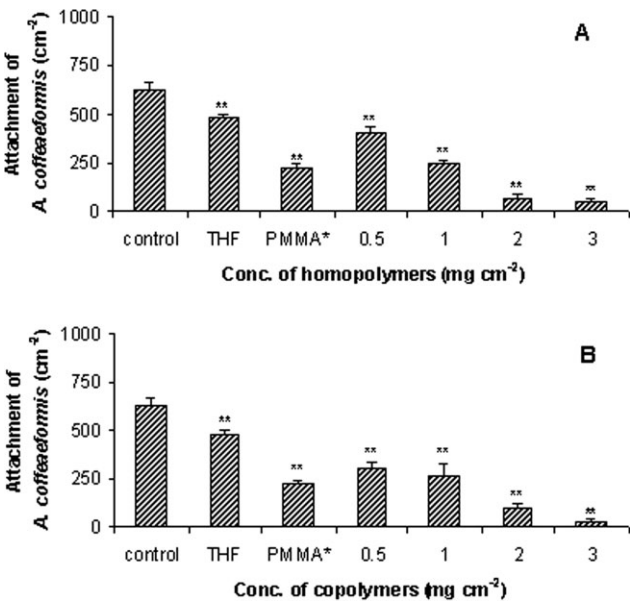

Figure 6 *Amphora coffeaeformis* attachment on polymer coatings. A: poly(AcBA); B: poly(AcBA-*co*-MMA). *PMMA, poly(methyl methacrylate): 1 mg cm^{-2}. Asterisks indicate mean values that are significantly different from control at *$P < 0.05$; **$P < 0.01$ (one-way ANOVA followed by Dunnett's one-tailed test).

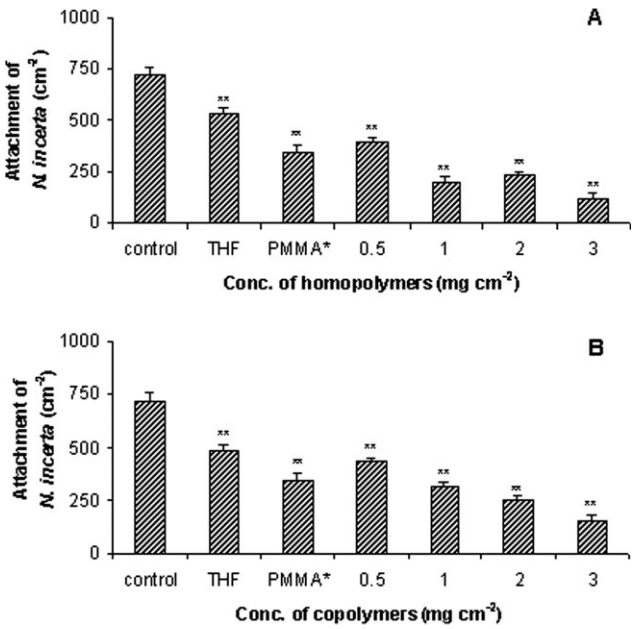

Figure 7 *Navicula incerta* attachment on polymer coatings. A: poly(AcBA); B: poly(AcBA-*co*-MMA). *PMMA, poly (methyl methacrylate): 1 mg cm^{-2}. Asterisks indicate mean values that are significantly different from control at *$P <$ 0.05; **$P < 0.01$ (one-way ANOVA followed by Dunnett's one-tailed test).

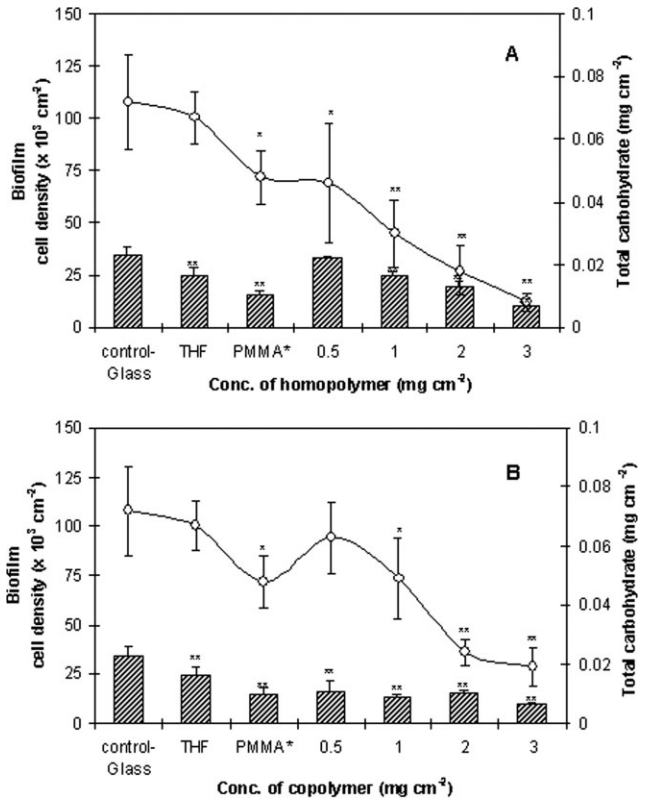

Figure 8 Biofilm formation of *Amphora coffeaeformis* on polymer coatings. A: poly(AcBA); B: poly(AcBA-*co*-MMA). *PMMA, poly(methyl methacrylate): 1 mg cm^{-2}. Asterisks indicate mean values that are significantly different from control at $^*P < 0.05$; $^{**}P < 0.01$ one-way ANOVA followed by Dunnett's test (biofilm cell density: one-tailed; total carbohydrate content: two-tailed).

contents, the total carbohydrate levels also increased in biofilms of *A. coffeaeformis*. The total carbohydrate contents of biofilms were proportionate to respective cell densities of *N. incerta* (Figure 7). The AcBA polymers coating of 2–3 mg cm^{-2} effectively prevented the biofilm formation of diatoms (Dunnett's test, $P < 0.01$).

Considering the controlled depletion of polymer coatings, currently AF polymers are largely preferred in AF paint formulations. In a previous study, the antimicrobial polymers were prepared with benzoicacid derivatives and their effectiveness against *Staphylococcus aureus* (ATCC 6538P), and *Pseudomonas aeruginosa* (ATCC 15522) were demonstrated.[10] Similarly poly(*N*-acryloyl-2-(4′-thiazolyl) benzimidazole) [poly(AcTBZ)] prepared with MMA by solution polymerization were reported[9] to be effective against microbes. In the present study, AcBA polymers (3 mg cm^{-2}) were found to effectively prevent the attachment of microfouling marine bacteria and diatoms. The effectiveness of AcBA can be comparable with the previous studies.[4,5] The AF potential of AcBA polymer coatings can be further increased by increasing the proportion of AcBA polymer in paint formulations.

Antibacterial properties of various benzaldehydes have been reported.[13,14,24,25] Antibacterial and antifungal properties of many essential oils have been attributed to one of their major constituents, benzaldehyde.[24,26,27] Based on the previous studies HBA was used in the present study to synthesize AF polymer. Moreover the comparison of benzaldehyde, benzoic acid and benzoic ester revealed that the benzaldehyde has strong antibacterial effects than others.[14] The results of the inhibitory concentrations of benzaldehyde (at 2 mg) obtained in the present study are in agreement with aforementioned findings. However, the number of sulfhydryl (—SH) groups present in the cell surface was reported to influence the antibacterial potential of the benzaldehyde.[14]

In a biotranformation study, bezaldehyde effect on biofilms of *Zymomonas mobilis* was studied by using a packed-bed column reactor.[28] In *Z. mobilis* biofilms treated with 30 m*M* of benzaldehyde for a short

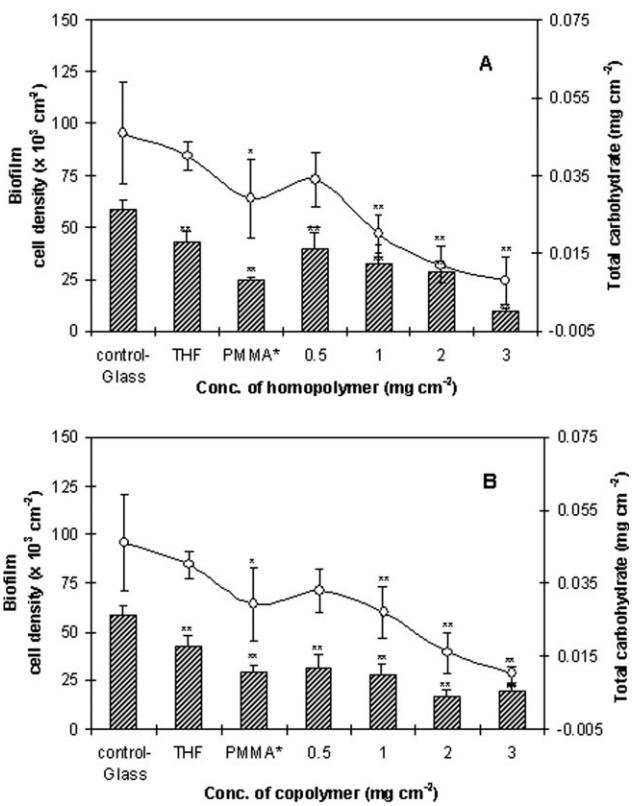

Figure 9 Biofilm formation of *Navicula incerta* on polymer coatings. A: poly(AcBA); B: poly(AcBA-*co*-MMA). *PMMA, poly(methyl methacrylate): 1 mg cm^{-2}. Asterisks indicate mean values that are significantly different from control at $^*P < 0.05$; $^{**}P < 0.01$ one-way ANOVA followed by Dunnett's test (biofilm cell density: one-tailed; total carbohydrate content: two-tailed).

period of 3 h, the metabolic activity of biofilms was decreased to 35%; however in planktonic forms it further reduced to a minimum of 10%. Similarly, in the present study also diatom biofilms were inhibited by AcBA.

p-hydroxybenzaldehyde is lignin associated substance derived from fungal decomposition of lignin.[29] In previous studies it has been reported from the decomposing barley straw,[30] rice straw[31] and in volatile compounds secreted by the oyster mushroom.[32] Allelochemical effects of these naturally occurring forms of *p*-hydroxybenzaldehyde were reported to be inhibitory against Chinese vetch, *Astragalus sinicus*,[31] bacteria,[32] blue green algae,[30,33,34] and diatoms.[35] The aforementioned studies clearly indicate the effective inhibitory role of *p*-hydroxybenzadehyde as evidenced from the present investigation against microfouling bacteria and diatom species. In a quantum structure-activity relationships (QSAR) study, Dai et al. reported the toxicity of 14 substituted benzaldehydes against *Daphnia magna*.[36] In which *p*-hydroxybenzaldehyde was found to be moderately toxic with a log EC_{50} (mol L^{-1}) value of −3.529. It was also found to be toxic only at high concentrations ($\geq$1000 μM) to the phytoplanktons.[37] The AF potential of benzoic acid and sodium benzoate against freshwater bacterial attachment was evaluated (Haque et al., 2005) with antifoulants entrapped in silicone coatings. They reported a 41–52% reduction in bacterial attachment mainly due to repulsive action of benzoic acid and sodium benzoate. This repulsion mechanism was ascribed to blocking of the specific binding sites or interfering with internal metabolism of the cells (Haque et al., 2005). It was also reported that benzoic acid inhibit the active uptake of some amino and oxo acids in bacterial cells (Russell and Chopra, 1996). In line with previous studies (Russell and Chopra, 1996; Vetere et al., 1999; Haque et al., 2005), at 0.5–3.0 mg cm^{-2} of AcBA polymer coatings, bacteriostatic or algaestatic effects were observed against marine bacteria and diatoms respectively, rather than killing them.[38–41] Conventionally, benzaldehyde-containing natural tannins (extracted from tree barks) have long been used to protect ship hulls from fouling organisms. A sodium salt of benzoic acid, sodium benzoate incorporated soluble matrix paint has been found to be effective in preventing barnacle and tube building amphipods settlement (Vetere et al., 1999). The narcotic effect of benzoate was attributed to repulsion of barnacle larvae (Vetere et al., 1999; Perez et al., 2001). A similar nontoxic AF repelling action is expected for AcBA polymers coatings. The hydrolysis products of AcBA polymers (benzoic acid, benzoate etc.) are relatively nontoxic and may not affect nontarget species; and pose adverse ecological impacts. Moreover, benzaldehyde and its metabolic intermediates have been found to biodegrade rapidly in the water column.[37,42,43] Therefore, it has comparatively less environmental impacts. Further studies are in progress to elucidate the interaction of —SH in the nontoxic AF mechanism of benzaldehyde against marine microfouling organisms.

CONCLUSIONS

The vinyl group functional monomer, AcBA were synthesized and identified by UV, IR, GC-MS, and ^{1}H-NMR subsequently homo- and co-polymers of AcBA were synthesized with MMA by solution polymerization. The number average (M_n) and weight average (M_w) molecular weights of the homo polymer were 84,400 and 142,300. The number average (M_n) and weight average (M_w) molecular weights of copolymer were 50,900 and 98,500, respectively. The antibacterial, diatom attachment and biofilm formation assays carried out on the homo- and co-polymers of AcBA, which showed potential AF activity against microfoulers. The poly(AcBA) coating was found to be effective in preventing the attachment and biofilm formation of diatoms at 3 mg cm^{-2} levels.

The authors thank Mr. Suh Ki Hyung for getting NMR data.

References

1. Kenawy, E. R. J Appl Polym Sci 2001, 82, 1364.
2. Tan, S.; Li, S.; Shen, S.; Liu, S.; Zong, M. J Appl Polym Sci 2000, 77, 1869.
3. Kenawy, E. R.; Abdel-Hay, F. I.; El-Shanshoury, A. E. R. R.; El-Newehy, M. H. J Polym Sci Part A: Polym Chem 2002, 40, 2384.
4. Park, E. S.; Moon, W. S.; Song, M. J.; Kim, M. N.; Chung, K. H.; Yoon, J. S. Int Biodeter Biodegr 2001, 47, 209.
5. Oh, S. T.; Ha, C. S.; Cho, W. J. J Appl Polym Sci 1994, 54, 859.
6. Pittman, C. U., Jr. J Coat Technol 1976, 48, 31.
7. Pittman, C. U.; Stahl, G. A.; Winters, H. J Coat Technol 1978, 50, 49.
8. Pittman, C. U.; Stahl, G. A. J Appl Polym Sci 1981, 26, 2403.
9. Oh, S. T.; Min, B. K.; Ha, C. S.; Cho, W. J. J Appl Polym Sci 1994, 52, 583.
10. Azab, M. M. Polym Int 2005, 54, 161.
11. Bringmann, G.; Kuhn, R. Water Res 1980, 14, 231.
12. Borneman, W. S.; Akin, D. E.; Van Eseltine, W. P. Appl Environ Microbiol 1986, 72, 1331.
13. Ramos-Nino, M. E.; Ramirez-Rodriguez, C. A.; Clifford, M. N.; Adams, M. R. J Appl Bacteriol 1998, 84, 207.
14. Friedman, M. L.; Henika, P. R.; Mandrell, R. E. J Food Prot 2003, 66, 1811.
15. Soler-Serratosa, A.; Kokalis-Burelle, N.; Rodriguez-Kabana, R.; Weaver, C. F.; King, P. S. Nematropica 1996, 26, 57.
16. Sethuraman, A.; Akin, D. E.; Eisele, J. G.; Eriksson, K. E. L. Can J Microbiol 1998, 44, 872.
17. Olivia, J. M.; Ballesteros, I.; Negro, M. J.; Manzanares, P.; Cabanas, A.; Ballesteros, M. Biotechnol Prog 2004, 20, 715.

18. Schrader, K. K.; De Regt, M. Q.; Tidwell, P. R.; Tucker, C. S.; Duke, S. O. Bull Environ Contam Toxicol 1998, 60, 651.
19. Shin, H. S.; Rogers, P. L. Appl Microbiol Biotechnol 1995, 44, 7.
20. Guillard, R. R. L.; Ryther, J. H. Can J Microbiol 1962, 8, 229.
21. McCoy, W. F.; Bryers, J. D.; Robbins, J.; Costerton, J. W. Can J Microbiol 1981, 27, 910.
22. Cole, B. E.; Thompson, J. K.; Cloern, J. E. Mar Biol 1992, 113, 219.
23. Dubois, M.; Gillies, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Anal Chem 1956, 28, 350.
24. Harbone, J. B. Plant Phenolics; Academic Press: London, 1989; p 23.
25. Ramos-Nino, M. E.; Clifford, M. N.; Adams, M. R. J Appl Bacteriol 1996, 80, 303.
26. Pawar, V. C.; Thaker, V. K. Mycoses 2006, 49, 316.
27. Prabuseenivasan, S.; Jayakumar, M.; Ignacimuthu, S. BMC Complement Altern Med 2006, 6, 39.
28. Li, X. Z.; Webb, J. S.; Kjelleberg, S.; Rosche, B. Appl Environ Microbiol 2006, 72, 1639.
29. Kivaisi, A. K.; Opden-Camp, H. J. M.; Lubberding, H. J.; Boon, J. J.; Voyels, G. D. Appl Environ Biotechnol 1990, 33, 93.
30. Everall, N. C.; Lees, D. R. Water Res 1997, 31, 614.
31. Pramanik, M. H. R.; Minesaki, Y.; Yamamoto, T.; Matsui, Y.; Nakano, H. Weed Biol Manag 2001, 1, 133.
32. Beltran-Garcia, M. J.; Estarron-Espinosa, M.; Ogura, T. J Agric Food Chem 1997, 45, 4049.
33. Everall, N. C.; Lees, D. R. Water Res 1996, 30, 269.
34. Newman, J. R.; Barrett, P. R. F. J Aquat Plant Manage 1993, 31, 203.
35. Barrett, P. R. F.; Curnow, J. C.; Littlejohn, J. W. Hydrobiologia 1996, 304, 307.
36. Dai, J.; Jin, L.; Yao, S.; Wang, L. Chemosphere 2001, 42, 899.
37. Means, J. L.; Anderson, S. J. Water Air Soil Pollut 1981, 16, 301.
38. Vetere, V.; Perez, M.; Garcia, M.; Deya, M.; Stupak, M.; Del Amo, B. Surf Coat Int 1999, 82, 586.
39. Perez, M.; Garcia, M.; Vetere, M.; Deya, M.; Del Amo, B.; Stupak, M. Pigment Resin Technol 2001, 30, 34.
40. Haque, H.; Cutright, T. J.; Newby, B. Z. Biofouling 2005, 21, 109.
41. Russell, A. D.; Chopra, I. Understanding Antibacterial Action and Resistance; Ellis Horwood: London, 1996; Chapter 3
42. Kota, S.; Borden, R. C.; Barlaz, M. A. FEMS Microbiol Ecol 1999, 29, 179.
43. Kamada, F.; Abe, S.; Hiratsuka, N.; Wariishi, H.; Tanaka, H. Microbiology 2002, 148, 1939.