Journal of Agricultural and Marine Sciences Vol. 20 (2015): 30-33.
Reveived 5 Aug. 2014
Accepted 19 Feb 2015

RESEARCH PAPER

Microfouling on biocidal and non-biocidal antifouling coatings

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التراكم الحيوي للكائنات الدقيقة على الطلاءات القاتلة وغير القاتلة المضادة للتراكم على الأسطح المغمورة

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Abstract. Although antifouling marine paints have been used to prevent biofouling, not much is known about their effectiveness in preventing attachment of microorganisms. The current study aims at estimating the abundance of bacteria within biofilms developed on various commercial antifouling coatings in Marina Bandar Rowdha and Marina Shangri La, Oman. Coatings tested included Pettit #1863 and #1792, West Marine #11046620, #5566252 and #10175206, Hempel Hard Racing #76484, Hempel Olympic #86950, Hempasil X3 and International YBA920. All coatings were applied on clean plastic slides. Slides without any coating were used as controls. Microbial biofilms were harvested after 2, 7 and 14 days of biofouling. Bacterial density was estimated using epifluorescence microscopy. There was a significant difference between the various treatments (coatings and control) after 2, 7 and 14 days of biofouling. Although there were significant differences between both locations after 2 and 14 days of biofouling, no significant difference was observed after 7 days of biofouling at both locations. At Shangri La, the lowest bacterial density was found on International YBA920, Pettit #1792 and Hempasil X3 after 2 days, 7 days and 14 days respectively in comparison to the control treatments. However at Bandar Rowdha, International YBA920 showed the lowest bacterial density after 2 days while West Marine #10175206 showed the lowest bacterial density after both 7 days and 14 days of biofouling in comparison to the control treatment. The differential performance of tested antifouling coatings may be attributed to several factors including varying environmental conditions, difference in microfouling communities, time of exposure and physical and chemical properties of antifouling coating.

Keywords: Coatings, antifouling, microfouling, bacteria, phototrophic.

المستخلص: بالرغم من أن الأصباغ البحرية المضادة للتراكم على الأسطح المغمورة تستخدم للحد من التراكم الحيوي ، إلا أنه لا يعرف الكثير عن مدى كفاءتها في الحد من تعلق الكائنات الدقيقة. وتهدف الدراسة الحالية إلى تقدير تواجد البكتريا في أغشية حيوية رقيقة تكونت على طلاءات تجارية مختلفة مضادة للتراكم على الأسطح في مارينا بندر الروضة ومارينا شانجري لا بسلطنة عمان. الطلاءات الي تم اختبارها شملت: ١٨٦٣ # Pettit و# ١٧٩٢ ، West Marine #١١٠٤٦٦٢٠ و ٥٥٦٦٢٥٢ #و #١٠١٧٥٢٠٦ ، Hempel hard Racing #٧٦٤٨٤ ، Hempel Olympic #٨٦٩٥٠ ، Hempasil X٣ ، International YBA٩٢٠. جميع الطلاءات تم وضعها على شرائح بلاستيكية نظيفة مع الإبقاء على بعض الشرائح غير مطلية لاستخدامها كمعاملة مرجعية. ومن ثم تم جمع الأغشية الحيوية الرقيقة المتكونة بعد ٢ و ٧ و ١٤ يوما من بداية التراكم الحيوي على الأسطح. وتم تقدير كثافة البكتريا باستخدام تقنية المجهر الفوق فلورسي. أظهرت النتائج وجود فروقات معنوية كبيرة بين المعاملات المختلفة (المطلية وغير المطلية) بعد ٢ و ٧ و ١٤ يوما من بداية التراكم الحيوي على الأسطح .وبالرغم من الاختلاف المعنوي في النتائج بين الموقعين بعد ٢ و ١٤ يوما من التراكم الحيوي على الأسطح ، إلا أنه لم يكن هناك فرق يذكر بعد ٧ ايام من التراكم الحيوي في الموقعين. وفي مارينا شانجري لا، كانت أقل كثافة للبكتريا في المعاملات: International YBA٩٢٠ و Pettit١٧٩٢# و Hempasil X٣ بالمقارنة مع المعاملات الغير مطلية، بعد ٢ و ٧ و ١٤ يوما على التوالي. أما في مارينا بندر الروضة فقد أظهر الطلاء International YBA٩٢٠ أقل كثافة للبكتريا بعد يومين من بداية التراكم الحيوي على الأسطح. بينما أظهر الطلاء West Marine #١٠١٧٥٢٠٦ أقل كثافة للبكتريا بعد٧ و ١٤ يوما مقارنة بالمعاملات الغير مطلية. إن الأداء المتباين للطلاءات المضادة للتراكم الحيوي التي تم اختبارها يمكن أن ينسب إلى عدة عوامل منها الظروف البيئية المختلفة، واختلاف مجتمعات المتراكمات الحيوية الدقيقة، ومدة التعرض، والخصائص الكيميائية والفيزيائية للطلاءات المضادة للتراكم على الأسطح.

الكلمات المفتاحية: الطلاءات ، مضادات التراكم الحيوي على الأسطح ، التراكم الحيوي للكائنات الدقيقة، البكتريا، الإنجذاب للضوء

Introduction

Biofouling has been a major problem in several marine industries including the shipping industry. Hull fouling adversely affects hydrodynamic drag leading to elevated fuel consumption and higher maintenance costs although the impact of biofilms is significantly less than that of macrofouling (Yebra *et al.* 2004; Schultz 2007; Schultz *et al.* 2011). In marine environments, formation of biofilms (ie microfouling) depends on the types of fouling microorganisms, environmental factors such as current, temperature, salinity, nutrient levels and hydrodynamic conditions (Wieczorek and Todd 1998 ; Lau *et al.* 2005; Zhang *et al.* 2011) and properties of substratum (Whitehead and Verran 2008). Mi-

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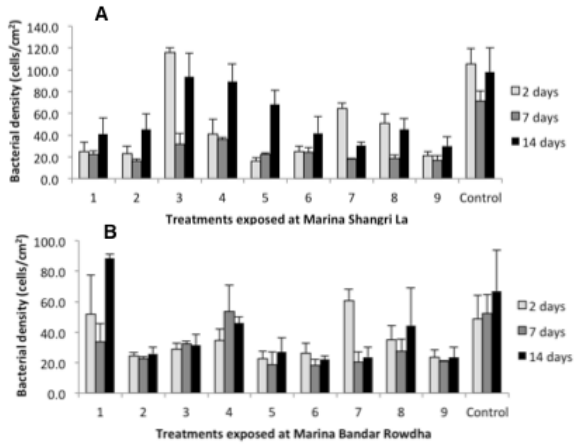

Figure 1. Bacterial density in biofilms developed on all treatments (Coatings 1-9 and Control) after 2, 7 and 14 days of biofouling at (**A**) Marina Shangri La and (**B**) Marina Bandar Rowdha . Data are the means + SD (n=3).

crobial fouling communities consist mainly of numerous species of bacteria and diatoms that can positively and/or negatively interact with each other (Railkin 2003; Dobretsov 2010). Both bacteria and diatoms may also have a significant impact on the recruitment of invertebrate larvae and algal spores (macrofouling) by either enhancing or inhibiting their settlement (Mitchell and Maki 1988; Maki 2002; Huang and Hadfield 2003; Qian *et al.* 2007; Hadfield 2011). This significantly influences the extent to which biofouling occurs in the marine environment. However bacteria have generally been accepted to be the primary colonizers on man-made surfaces in the marine environment (Molino *et al.* 2009b). Therefore it is important to study the efficiency of antifouling coatings in preventing bacterial fouling during the primary stages of biofouling in the marine environment. The objective of the current study was to estimate the abundance of bacteria within biofilms developed on various commercial antifouling coatings at two different locations in Oman. The hypothesis tested was that treatments (nine commercial antifouling coatings) and location influence the abundance of bacteria within biofilms developed on commercial antifouling coatings.

Materials and methods

Coatings preparation

Six commercial antifouling coatings (Petit # 1863, Petit 1792, West Marine #5566252, West Marine #11046620, West Marine #10175206 and International Micron Extra YBA 920) were obtained at local boat shop (Muscat, Oman). Three commercial antifouling coatings (Hempel Hard Racing 76484-51170, Hempel Olympic 86950-5110 and Hempasil X3) were obtained from Hempel Ltd. Co. (Muscat, Oman). The nine antifouling coatings (Table 1) were manually applied onto cleaned, acrylic plastic slides (75 x 25 mm) at Marine Science and Fisheries Laboratory, Sultan Qaboos University, Oman. All coated slides were dried for several hours at ambient temperature prior to deployment. Uncoated cleaned plastic slides were considered to be the control treatments. For each treatment including control, a total of 18 replicate slides were prepared.

Coatings Deployment

A total of 180 slides were randomly inserted into 6 slide cassettes (each 21 x 16 x 3 cm) such that each slide cassette contained 3 replicates of each treatment and 30 equally spaced slides in total. Each slide cassette was deployed by ropes such that each slide in the slide cassette was kept vertical with respect to the surface of seawater. Three slide cassettes were deployed each at Marina Shangri La (Muscat, Oman 23° 32' 55" N 58° 39' 23" E) and Marina Bandar Rowdha (Muscat, Oman 23° 34' 55" N 58° 36' 27" E).

Sample collection

Each of the three slide cassettes at Marina Bandar Rowdha and Marina Shangri La were withdrawn after 2 days, 7 days and 14 days of biofouling respectively. During sample collection, all slides from the slide cassette were carefully transferred into clean plastic boxes containing formalin (3.7% final concentration) and immediately transferred to the laboratory at 4°C for further analysis (see below).

Estimating abundance of bacteria

The total bacterial density on the treatment surfaces was estimated by staining an area of 2 x 2 cm with 10-12 µl of 4, 6-diamidino-2-phenylindole (DAPI, Sigma, Germany) solution for 15 minutes according to Dobretsov and Thomason (2011). The number of bacteria in 10 randomly selected fields of view on the ocular grid (0.001 mm^2) was counted using an epifluorescence microscope (Axiostar plus, Zeiss, Germany; magnification 1000x; λ_{Ex}=359nm, λ_{Em}=441nm).

Statistical analysis

Factorial ANOVA was used to test the effect of treatment and location on the total bacterial density using Statistica 11 (Statsoft, USA) after 2, 7 and 14 days of biofouling. *Post hoc* HSD test was used to test for significant differences among the treatments and locations. In all cases, the threshold for significance was 0.05.

Results

The treatments (antifouling coatings and control) significantly influenced the bacterial density in biofilms developed after 2, 7 and 14 days of biofouling (Figure 1A and Figure 1B; ANOVA, HSD, $P < 0.0001$). Although

Table 1. Characteristics of the ten treatments exposed to biofouling at depth, 1m at Marina Shangri La and Marina Bandar Rowdha.

Treatment	Commercial coating	Type of coating	Active Ingredient
1	Petit Marine #1863	Biocidal	Zinc pyrithione
2	Petit Marine #1792	Biocidal	Pure Zinc
3	West Marine #5566252	Biocidal	Cuprous Thiocyanate
4	West Marine #11046620	Biocidal	Zinc pyrithione
5	International micron extra YBA920	Biocidal	Cuprous oxide + Dichlofluanid
6	West Marine #10175206	Biocidal	Cuprous oxide
7	Hempel Hard Racing #76484-51170	Biocidal	Cuprous oxide
8	Hempel Olympic #86950-51110	Biocidal	Copper
9	Hempasil X3	Non-biocidal	Silicone
Control	-	-	-

both locations were found to significantly affect bacterial density after 2 and 14 days (ANOVA, HSD, $P < 0.0001$) there was no significant difference between both locations after 7 days of biofouling (ANOVA, HSD, $P = 0.237$). However both treatments and locations together significantly affected the bacterial density in biofilms after 2, 7 and 14 days of biofouling (ANOVA, HSD, $P < 0.01$). At Shangri La, the lowest bacterial density was found on International YBA920, Pettit #1792 and Hempasil X3 after 2 days, 7 days and 14 days respectively in comparison to the control treatments (Figure 1A). However at Bandar Rowdha, International YBA920 showed the lowest bacterial density after 2 days while West Marine #10175206 showed the lowest bacterial density after both 7 days and 14 days of biofouling in comparison to the control treatment (Figure 1B). The differential performance of tested antifouling coatings may be attributed to several factors including varying environmental conditions and differences in the abundance of fouling bacterial communities. The variation in the concentrations of biocides in these coatings may be additional factor in influencing bacterial attachment on coatings. In particular the polishing rate behavior and biocide delivery rate behavior is known to vary for different coating types (Finnie & Williams 2010, Bressy *et al.* 2010). Clearly further investigations are required to study the abundance and composition of bacterial fouling communities on antifouling coatings.

Conclusions

The current study shows that the abundance of bacteria in biofilms developed on commercial antifouling coatings is significantly influenced by the coating types and both coatings and location together after 2, 7 and 14 days of biofouling. Varying environments were not found to affect the bacterial density after 7 days of biofouling although there were significant differences after 2 and 14 days of biofouling.

Acknowledgements

This study was supported by HM Sultan Qaboos Research Trust Fund SR/AGR/FISH/10/01. The authors thank Hempel Ltd. Co. (Muscat, Oman) for providing coatings. The authors would also like to thank Ms. Annika Vaksmaa (Sultan Qaboos University) for her assistance in experimental setup.

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