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Impact of wood preservative on the primary film formation in rubber wood

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Abstract

The adhesion of bacterial cells onto biocidal coating (boric acid) of rubber wood coupons in the marine environment upto 24 hours was investigated in the present study. Gram negative bacteria dominated both the untreated and treated wood of which *Pseudomonas* sp. was found to be the dominating species. The results showed that the biofilm formation was found to be reduced in treated wood when compared to untreated wood. It may be due to the leached components of the preservative boric acid in the treated wood.

Keywords

Biofilm, boric acid, leaching

Introduction

Biofilms are defined as microbial communities of cells that are irreversibly attached to a substratum, to an interface, or to each other and are embedded into a matrix of extracellular polymeric substances that they have produced (Donlan and Costerton, 2002). Biofilm formation is the preliminary stage in the process of biofouling on submerged objects in the marine environment (Maki, 1999). Marine biofilms are ensembles of organic molecules, microalgae, bacteria and fungi (Wahl, 1989).

Materials immersed in seawater are acted upon by a series of physical, chemical and biological events which result in the formation of a biofilm complex depending on the polluted nature of the environment (Srivastava *et al.*, 1990 and Abarzua and Jakubowski, 1995). The bacterial biofilm changes the topography and chemistry of the surface. A number of other microorganisms including fungi, diatoms, cyanobacteria and microalgae and invertebrates settle and attach to the substance to form a complex structure known as biofouling (Callow and Callow, 2002).

Fouling organisms are known to cause serious problems by settling on man-made structures such as ship hulls, cooling system pipes of powerstations and other maritime industries and affect aquaculture nets by increasing the hydrodynamic drag and increasing the expenses for cleaning (Lewis, 1994). To prevent damage, wood is treated with preservatives which inhibit the settlement and growth of fouling organisms (Srinivasan and Swain, 2007). It has been demonstrated that they leach from the treated wood when it is submerged in marine ecosystem (Breslin and Adler-Ivanbrook, 1998) and influence the biofilm formation (Kropfl *et al.*, 2006). Hence, in the present study, regular observation was carried out on both boric acid treated and untreated wood for a period of 24 hours and the effect on bacterial count was studied after determined exposure periods.

Materials and methods

Preparation of wood coupon and bacterial characterization

Both untreated wood and boric acid treated wood was cut into dimensions of 6×8 cm^2 and were suspended at a depth of 1m at Vattakottai sea, Kanyakumari district off the East coast of India during December, 2011. Biofilm sampling was made for a period of 24 hrs. Coupons were retrieved after 1, 3, 6, 9, 12, 15, 18, 21 and 24 hrs of exposure and brought to the laboratory in a box containing filtered (Millipore, 0.45µm) seawater.

In the laboratory, the retrieved coupons were carefully rinsed with filtered sea water (Millipore, 0.45µm) to remove unattached microbes. The biofilm was scrapped off using a sterile nylon brush and the material was dispersed in filtered and sterilized sea water and was used for the study of microfouling community (Satheesh and Wesley, 2010).

Examination of bacterial colonies

For quantitative examination of bacterial colonies, the biofilm sample obtained above was serially diluted (10-fold) using filtered sterilized sea water and 0.1ml was plated on Zobell marine agar (Himedia, India) by pour plate method. The inoculated petridishes were incubated at room temperature (28 ± 2ÚC) for 24h and the total viable count was noted. Total bacterial count was expressed as CFU/cm^2.

For characterization of bacteria upto generic level, a loop full of bacterial culture was inoculated into sterile Zobell marine nutrient broth and incubated overnight. Bacterial isolates were identified using Bergey's Manual of Systematic Bacteriology, 9th edition (Holt *et al.,* 1994). T-test was applied to assess the difference in bacterial count on the primary film formation between treated and untreated wood.

Results and Discussion

The development of biofilm and fouling communities is a multiple event with numerous interactions taking place between fouling organisms colonizing the surface. The first colonizers on any newly exposed surface in marine waters are bacteria and they have been found to affect the subsequent recruitment of both microorganisms and macrofoulers (Kolappan and Satheesh, 2011). Surface charge plays an important role in the development of biofilm (Tsibouklis *et al*., 1999; Bhosle *et al*., 2005; Jain and Bhosle, 2009) serving to prevent or encourage the adhesion of microorganisms (Tsibouklis *et al.,*1999). Hence, control of biofilms on surfaces is an important strategy in any biofouling management programme. The results of the present study indicate that the viability of the bacterial count in boric acid treated wood was reduced considerably when compared to untreated wood. It was supported by the work done by Maliavski *et al*. (1995) who reported that biocidal fouling may also be reduced or delayed by a slow release of biocides.

In the present study the heterotrophic bacterial population ranged from 8×10^6 CFU /cm^2 to 145.25×10^6 in untreated wood whereas it ranged from 0.75×10^6 to 55.75×10^6 CFU/ cm^2 in the treated wood. Settlement of bacteria was observed within an hour of coupon exposure and their abundance increased with exposure time (Satheesh and Wesley, 2010). Daniel (1963) also reported that bacterial settlement occurred on glass coupons within an hour of exposure at Madras Coast (East coast of India).

Kanematsu *et al. (*2009) reported that the inhibition capability against biofilm formation decreased with the increase of immersion time which differed from specimen to specimen. Generally, the rates of metal loss from treated structures were reported to be highest soon after submersion in the seawater, which gradually decreased as time progressed. The impact of leachate was found to be higher on fouling organisms in confined conditions. But, in actual field conditions, the leached metals diluted immediately due to water mixing. In the present study t-test showed that there was significant difference in total bacterial count between untreated wood and treated wood ($t = 2.35$, $df=9$, $p<0.05$).

In the present study, both the untreated and treated wood coupons were dominated by Gram negative bacteria. The genera identified under Gram negative group were *Pseudomonas* sp., *Vibrio* sp., *E.coli, Aeromonas* sp., and Gram positive group were *Micrococcus* sp., *Bacillus* sp., *Staphylococcus* sp. Balasubramanian *et al*. (2012) observed that generic composition of the heterotrophic biofilm was found dominated by Gram-negative groups followed by Gram-

TABLE-1

VIABLE COUNT OF BACTERIAL POPULATION OBSERVED AFTER DIFFERENT HOURS OF IMMERSION PERIOD

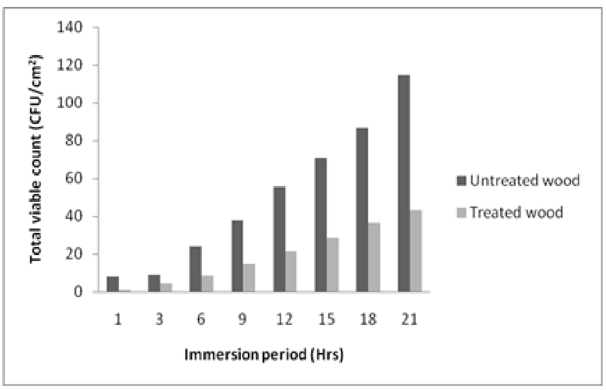
IMMERSION PERIOD (Hrs)	UNTREATED WOOD $\times10^6$(CFU/cm^2)	TREATED WOOD $\times10^6$ (CFU/cm^2)
1	8	0.75
3	19	4.25
6	24	8.25
9	37.75	14.5
12	55.5	21.5
15	70.5	28.25
18	86.75	36.5
21	114.75	43.25
24	145.25	55.75

TABLE-2

MICROBIAL COMMUNITY OF WOOD BIOFILM

Immersion period (Hrs)	Untreated wood	Treated wood
1	*Pseudomonas* sp., *Bacillus* sp.	*Pseudomonas* sp.
3	*Bacillus* sp., *Pseudomonas* sp., *Vibrio* sp.	*Pseudomonas* sp.
6	*Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp.	*Pseudomonas* sp.
9	*Vibrio* sp., *Bacillus* sp.	*Pseudomonas* sp., *Vibrio* sp.
12	*E. coli*, *Pseudomonas* sp., *Vibrio* sp.	*Pseudomonas* sp.
15	*Pseudomonas* sp., *Aeromonas* sp.	*Bacillus* sp., *Vibrio* sp.
18	*Pseudomonas* sp., *Micrococcus* sp., *Vibrio* sp.	*Pseudomonas* sp., *Micrococcus* sp.
21	*Bacillus* sp., *Staphylococcus* sp.	*Bacillus* sp., *Vibrio* sp.
24	*Bacillus* sp., *Micrococcus* sp., *Pseudomonas* sp.	*Bacillus* sp., *Micrococcus* sp.

Fig.1 COLONY FORMING UNIT OF BIOFILM AFTER DIFFERENT HOURS OF IMMERSION



positive groups. Accordingly, in the present study *Pseudomonas* sp. was found to be the dominating species both in untreated and treated wood. Balasubramanian *et al.* (2012) reported that the Gram-negative bacteria *Pseudomonas* sp. was found to be the pioneer bacteria to colonize the surface of polyvinylchloride sheet. Flagellar motility and adhesion via pilli were important for initiating biofilm formation in *Pseudomonas* sp. under static conditions (O'Toole and Kolter, 1998). The lipopolyssacharide (LPS) of Gram negative bacteria was an added advantage for the bacterium to adhere and colonize (Davies *et al.*, 1998).

In the present study, *Pseudomonas* sp., *Micrococcus* sp., *Bacillus* sp., *Staphylococcus* sp., *Vibrio* sp., *E. coli, Aeromonas* sp. were found to inhabit the untreated wood biofilm whereas *Pseudomonas* sp., *Vibrio* sp., *Bacillus* sp., *Micrococcus* sp., were found to inhabit treated wood biofilm (Table-2). Leached metals from treated wood were a source of pollution to marine biota which resulted significantly in less bacterial count. The total bacterial count was actually low in boric acid treated wood when compared to untreated wood. This suggested that leached components of the preservative had adverse effect on biofilm forming organisms.

Conclusion

The leached components of the preservative, boric acid in the treated wood had an adverse effect on the biofilm forming bacteria upto 24 hrs.

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