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Full Length Research Paper

Novel approach for quantitative estimation of antifouling activity in marine paint

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Biofouling and biofilm formations are of great concern to many modern industries, including marine, food, water, mining and medical field. The shipping industry has serious problems with biofouling (complex communities of marine organisms) on most surfaces submerged in seawater. This leads to problems such as increasing water resistance, fuel consumption and microbial corrosion of metal surfaces. The primary fouling agents on aquatic surfaces exposed to light happen to be algae and diatoms, whereas fungi and bacteria follow the trend, forming a biofilm on the surface. This further attracts the crustaceans and molluscs, which are responsible for macro-fouling. Various components such as tin, arsenic, mercury, lead and copper are used as the biocides. These components are polluting the environment affecting the marine life. The paper contents the use of herbal extract as the antifouling agent and approach for quantitative estimation of the antifouling activity of the *Curcuma longa* extract as compare to that of tin.

Key words: Antifouling, *Curcuma longa*, leaching, paint, *Gleocapsa*.

INTRODUCTION

Biofouling and biofilm formations are of great concern to many modern industries, including marine, food, water, mining and medical field. The shipping industry has serious problems with biofouling (complex communities of marine organisms) on most surfaces submerged in seawater because of increasing water resistance, fuel consumption and microbial corrosion of metal surfaces. The economic consequence of biofouling is thus significant.

Biofouling is defined as undesirable changes, brought about by the living organisms in the quality of value of the material, either in aesthetic or utilitarian terms. The course of fouling and the microorganism involved in fouling varies according to the location and the environment. The primary fouling agents on aquatic surfaces exposed to light happen to be algae and diatoms, whereas fungi and bacteria follow the trend, forming a biofilm on the surface. This further attracts the crustaceans and molluscs, which are responsible for macrofouling.

In ancient days various compounds such as salts of mercury, arsenic, lead, copper have been tried as biocides, which dissolved slowly and released toxic component in the surroundings. These biocides often showed marginal effect, low cost effectivity. The introduction of tributyl tin as a biocide in 1960 appeared to solve all the problems. Paints with TBT appeared (at least, for a while) to provide the ideal answer to fouled hulls. The tin-based compound could be dissolved directly in the film-forming paint rather than being dispersed as an insoluble powder. The resulting paints were not only excellent antifouling coatings, but were colorless, permitting for the first time the introduction of bright-colored bottom paints. These paints didn't have to be loaded with heavy metallic dust, and therefore were easier to apply. Tin-based paints also could be applied months before launching, and could provide multi-year protection even when the boat was hauled at the end of the season. By the end of the 1960's, TBT paints dominated the field. However TBT was not only effective in killing growth on the bottom of a boat, but it was doing the same thing to fish and shellfish in the surrounding waters. It's very difficult to create a "non-toxic biocide." When that biocide is expected to control the huge variety of organisms that contribute to fouling, the task is not just difficult; it's nearly impossible.

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In order to solve this problem, it is necessary to find antialgal substances without toxicity or with very low toxicity to the non-target species. Many such ecofriendly biocides have been identified and reported. Preliminary studies carried out in this laboratory indicated algicidal activity in some essential oils as well as in hexane extracted solids from *C. longa*. (Chandak et al., 2003 unpublished data). However it is essential to compare the biocidal activity in quantitative terms in order to make a final selection.

Concept of Phenol coefficient for comparing antimicrobial activity of experimental compound with phenol as a standard is commonly employed in evaluation of disinfectants. It is defined as (Collins and Patricia, 1970).

$$\text{Phenol coefficient} = \frac{\text{Greatest dilution of the disinfectant killing the test organism in 10 min but not in 5 min}}{\text{Greatest dilution of the phenol killing the test organism in 10 min but not in 5min}}$$

Another concept for assessment of environmental toxicity is LD50 which is defined as the dose required to kill 50% of the exposed population (Ananthanarayanan and Jairampaniker, 1978) These two concepts are combined to coin the term "Tin coefficient which is defined as:

$$\text{Tin coefficient} = \frac{\text{Conc. of test material giving 50\% inhibition in 15 days}}{\text{Conc. of tin giving 50\% inhibition in 15 days}}$$

The investigation reported in this paper was carried out to study the Practical application of above concept for assessment of antifouling activity of selected acrylic paint containing *C. longa* extract solids.

C. longa which, belongs to family Zingiberaceae, is the traditional medicine of India. It was prescribed for treatment of many conditions; including poor vision rheumatic pains, and cough.

Native people of the pacific sprinkled the dust on their shoulder during ceremonial dances and used it for numerous medical problems ranging from constipation to skin diseases. It is also used in the intestinal infections (Gujaral et al., 1953).

Further the inhibitory effect of Curcuma oil against different microorganism like *Staphylococcus albus* etc were studied (Chopra et al., 1941). They found Curcuma oil highly effective antimicrobial agent. This was supported by a Reported MIC of 1 to 5000 against these microorganisms.

Active ingredients like Curcumin, ionone and turmeron was tested for antimicrobial activity against *Bacillus subtilis, Cornebacterium diptheriae, Staphylococcus aureus, Escherichia coli, Aspergillus etc*. These ingredients exhibited strong to moderate inhibitory action against the entire microorganism tested (Garg and Jain, 1998).

Turmeric leaf oil was found to be bactericidal to *Shigella* species at variable concentration of 7.81, 3.25, 62.5 µl/ml. The effect was immediately observed both at 37 and 4°C indicating temperature and time independent activity (Rath Chandi et al., 1999).

METHODOLOGY

The hexane extract of *C. longa* rhizome was prepared using Soxhlet extraction method. Hexane was evaporated and then a concentrated extract 16% solid was used for the further studies.

Growth curve of algae

The marine algae *Gleocapsa sp* BDU 48121 procured from National Facility for Marine Cyanobacteria, Tamilnadu, was used as a test organism. Growth curve of algae in ASN medium was established by standard method (Kaushik, 1987). Duration of the growth cycle of the *Gleocapsa sp*, was around 20 days with the maximum growth rate was observed up to 15 days. The time period for the further experiments was set as 15 days.

Paint formulations

Basic acrylic paint (P1) was modified by adding different levels of tin acrylate (P2-P4) and *C. longa* extract (P5-P8) as indicated in Table 2.

Leaching experiment

Beakers of 250 ml capacity were coated with test paints (Basic paint and paint with extract) at the inner surface. Initial weights of all the beakers were recorded and one uncoated beaker was kept as control. The beakers were filled with sterile ASN III medium (Medium specific for growth of algae) and covered with foil to avoid contamination and was kept for 15 days at ambient conditions. Medium was taken out in pre sterilized flasks under aseptic conditions.

The beakers were allowed to dry in the air. Fresh sterile ASN III medium was taken in these beakers and kept for next 15 days. Same procedure was repeated at the interval of 15 days for two months.

Antifouling activity of leachate

Medium transferred in pre-sterilized flask as mentioned in earlier step, was inoculated with *Gleocapsa sp.* culture of predetermined O.D. and kept at 28°C in proper illumination. Chlorophyll content was estimated on first, seventh, and fifteenth day (Kaushik, 1987).

Same procedure was repeated for two months at the interval of 15th day (Table 1). Effect of antifouling components on morphology of algae was studied under microscope. Percent inhibition was calculated as (Table 2).

$$\text{Percent inhibition} = 100 - \frac{\text{Chlorophyll in Experimental flask culture}}{\text{Chlorophyll in control flask culture}} \times 100$$

Table 1. Effect of leach water on growth of *Gleocapsa* sp.

Day of exposure	No. of days	Chlorophyll content (mg/ml)				% inhibition		
		Control	P1(pure)	P2(HH)	P11(L)	Pure	HH	L
LE –15	1st	0.1573±0.028	0.1573±0.028	0.1573±0.028	0.1573±0.028			
	7th	0.1864±0.018	0.0552±0.019	0.0420±0.014	0.0168±0.006			
	15th	0.2715±0.017	0.1301±0.018	0.078±0.019	0.069±0.018	100	100	100
Mean		0.205^{c}	0.1142^{b}	0.092^{ab}	0.08^{a}			
				F =9.19***				
LE-30	1st	0.0915±0.014	0.0915±0.014	0.0915±0.014	0.0915±0.014			
	7th	0.1052±0.011	0.0647±0.013	0.1025±0.025	0.0764±0.022			
	15th	0.1251±0.014	0.1081±0.018	0.1121±0.013	0.1025±0.023	50.6	38.6	67.2
Mean		0.107^{b}	0.08^{a}	0.1020^{ab}	0.09^{ab}			
				F=2.85**				
LE-45	1st	0.0422±0.010	0.0422±0.010	0.0422±0.010	0.0422±0.010			
	7th	0.0692±0.017	0.1242±0.008	0.0602±0.018	0.0942±0.009			
	15th	0.1141±0.020	0.2837±0.018	0.0874±0.018	0.1553±0.013	0	37.2	0
Mean		0.075^{ab}	0.1497^{b}	0.063^{a}	0.097^{ab}			
				F=3.47**				
LE-60	1st	0.0378±0.003	0.0378±0.003	0.0378±0.003	----			
	7th	0.0725±0.002	0.0892±0.003	0.0923±0.004	----			
	15th	0.1237±0.004	0.1357±0.010	0.1579±0.012	----	0	0	0
Mean								
				F= 0.35				

Mean in the same Row without a common superscript are significantly different. Values in columns are mean of triplicate ± SD..
***Significant at 1%, **Significant at 5%, *Significant at 10%.

Table 2. Effect of variation in concentration of active component on growth of *Gleocapsa* sp.

Active Component	1st day	7th day	15 days	Mean	
			Tin		
Control	0.2495±0.0195	0.4058±0.011	0.4796±0.008	0.3783^{b}	---
P1	0.2495±0.0195	0.2408±0.016	0.4598±0.0148	0.3167^{ab}	8.60
P2 (0.09%)	0.2495±0.0195	0.2646±0.0097	0.3962±0.0177	0.3034^{a}	38.85
P3 (0.18%)	0.2495±0.0195	0.3515±0.0087	0.2971±0.011	0.299^{a}	79.31
P4 (0.27%)	0.2495±0.0195	0.2798±0.0162	0.2777±0.011	0.269^{a}	87.74
			F=2.63**		
			Curcuma longa		
Control	0.2495±0.0195	0.4058±0.011	0.4796±0.008	0.3783^{b}	
P1	0.2495±0.0195	0.2408±0.016	0.4598±0.0148	0.3167^{ab}	
P5 (E) (0.08%)	0.2495±0.0195	0.2772±0.011	0.4835±0.027	0.367^{b}	-
P6 (E) (0.16%)	0.2495±0.0195	0.4567±0.007	0.3842±0.013	0.363^{b}	41.16
P7 (E) (0.24%)	0.2495±0.0195	0.3141±0.012	0.3515±0.0262	0.305^{ab}	55.67
P8 (E) (0.32%)	0.2495±0.0195	0.2537±0.0137	0.3392±0.0170	0.280^{a}	61.01
			F=2.36**		

Mean in the same column without a common superscript are significantly different. Values in columns are mean of triplicate ± SD
***Significant at 1%, **Significant at 5%, *Significant at 10%.

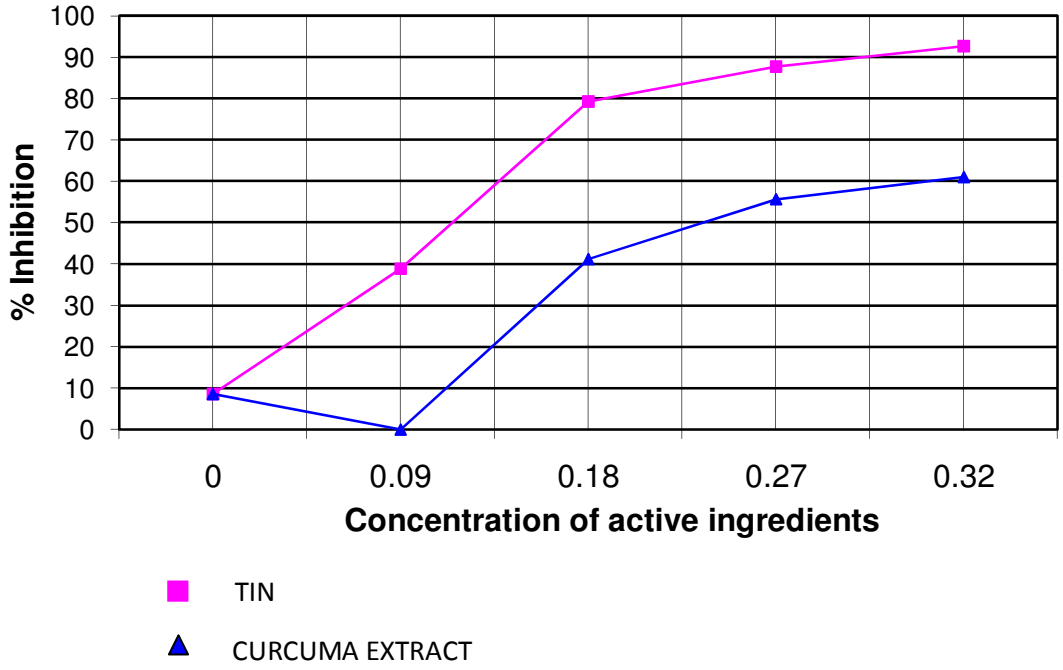

Figure 1. For tin coefficient.

Estimation of tin coefficient

The concept of phenol coefficient for testing of any disinfectant was considered as a base for proposed method for quantitative assessment of antifouling activity. As tin is considered as standard antifouling substance, antifouling activity was compared with tin.

Tin acrylate paint containing various levels of tin was prepared by blending plain acrylate and Tin acrylate (containing 4% tin) in different proportions. Second set of plain acrylate paint contained *C. longa* extract (16% solid) ranging between 0.50 to 2%.

Beakers were coated with all the above-prepared paints, filled with ASN III medium and covered with foil. After 15 days, medium was aseptically transferred to flasks and inoculated with the *Gleocapsa* sp. culture of predetermined O.D. Plain acrylate paint without any additive served as control.

Chlorophyll content was estimated at the end of seven and fifteen days and % inhibition was calculated. Graph of concentration against % inhibition was plotted. Concentration corresponding to 50% inhibition was calculated from the graph in both the cases (Table 2 and Figure 1).

"Tin coefficient" is defined as follows:

$$\text{Tin coefficient} = \frac{\text{Conc. of test material giving 50\% inhibition in 15 days}}{\text{Conc. of tin giving 50\% inhibition in 15 days}}$$

RESULTS AND DISCUSSION

Growth curve of algae indicated that *Gleocapsa* sp remained in Exponential phase upto 14 days. From the leaching experiment the result indicate that both the paints pure as well as paint containing extract show 100% inhibition of algae growth on 15 days exposure. The metallic pigment components of paints may be partly responsible along with the active ingredients.

After 30 days basic paint shows 50% inhibition whereas *C. longa* extract containing paint 38.9% inhibition. But at 45 days exposure basic paint shows 0% inhibition while paint containing *C. longa* extract shows 37.17% inhibition. This indicates better retention of active principle *C. longa* extract paint However all antialgal activity is lost at the end of 60 days in both paints.

Further in the experiment for calculation of tin coefficient, rate of leaching increases with increase in percentage of Tin as well as *C. longa hexane* extract solids (Table 2) incorporated in paint and so is the increase in inhibition. From the definition of the tin coefficient 50% inhibition was observed in the both the paints at the concentration0.12% of tin and 0.20% of *C. longa extract*.

Tin coefficient is calculated as:

$$\text{Tin coefficient} = \frac{\text{0.20\% } \textit{Curcuma longa} \text{ extract}}{\text{0.12\%Tin}}$$

Tin coefficient of *C. longa hexane* extract is 1.66.

Conclusion

From the above experiment it is concluded that the tin coefficient of the *C. longa hexane* extract is 1.66. Higher concentration of extract is probably required than tin due to higher molecular weight of natural components. It is

worthwhile to use natural biocide, in view of the environmental and economic advantages. The extract does not have any toxic effect on the marine life as that of tin.

This method can also be applied for testing of different antifouling agents and its efficiency can be tested using the above experiment.

The paint composition has excellent Drying, Hardness, gloss and resistance characteristics. The conventional properties, which are required basically for development of antifouling coatings, are already present in the formulation. The harbor site testing of final antifouling paint is also required and then only the paint can be taken on large scale

The tabulated data were statistically analyzed by carrying out a one way analysis of variance test (ANOVA) for significance (Snedecor and Cochran, 1967).

The results when analyzed statistically indicate that the activity in both the series of paint containing tin as well as *C. longa was* significant at 5% significance level.

The statistical analysis shows that in LE-15 that is, in first 15 days, ($F = 9.19^{***}$) is significant at 1% level. While LE-30 ($F = 2.85^{**}$) and LE-45 ($F=3.47^{**}$) which is significant at 5% level.

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