A laboratory bioassay for the efficacy of anti-fouling paints using *Ectocarpus siliculosus* (a preliminary report)

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1. Background

Translocation of invasive aquatic species through biofouling of ships or ballast water and sediments has been one of the serious problems among the marine environmental protection issues.

In order to develop more effective antifouling paints, it is very important to test and quantify the efficacy of new antifouling systems including antifouling agents. The authors have already reported test protocols for evaluating the efficacy of anti-fouling paints using the mussel[1] and barnacle[2]. The purpose of this study is to establish the test protocol using algae, from the viewpoint of broadening the fouling species accessible as test organism, and to propose an experimental design as an International Standard Test Protocol, such as ISO standards.

Reference: [1] R. Kojima et al., PLoS ONE, 2016. [2] R. Kojima et al., ICMCF satellite symposium , 2014.

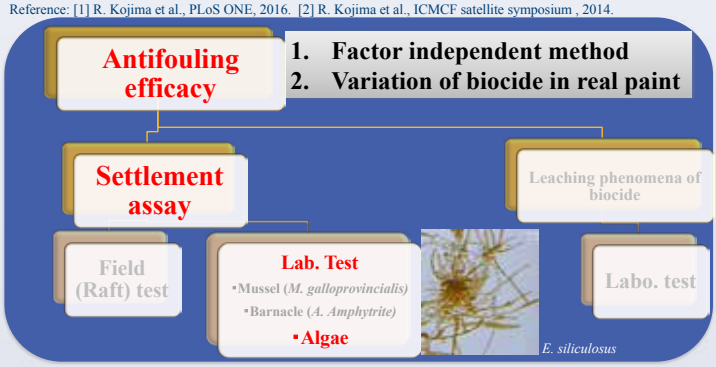


Figure 1. Establishment of the test protocol.

2. Experiment

2-1. Preparation of test paint

Test panels were prepared with different formulations of antifouling paints by varying the content of Cu_2O, the most commonly used antifouling agents, from 0 to 40 wt.%

Table 1. Preparation of test paint.

Entry	Cont.	1	2	3	4
Cu_2O content [wt%]	0(PVC)	0	5	20	40

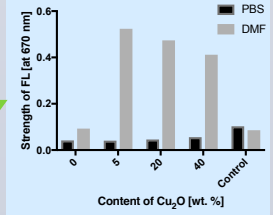
*) Hydration type copolymer (polyvinyl chloride and polyvinyl isobutyl ether)

2-2. Preparation of test panels in natural seawater (NSW) with dynamic conditions.

- 51 × 49 × 2.0^{t}mm (painted: 51 × 43mm, PVC panel)
  - ➢ Exchange NSW, below100ppb of Cu
  - ➢ 20℃, Ca. 10knot
  - ➢ 45 days

Figure 2. Schematic diagram of dynamic aging instrument with panels.

2-3. Extraction of Chlorophyll-a by a suitable solvent



DMF(*N,N*-dimethyl formamide) is suitable solvent for the extraction of *Chl-a*.

Figure 3. Comparison of FL strength between PBS and DMF.

2-4. Laboratory bioassay

The unialgal culture strain of a filamentous brown alga *E. siliculosus* (KU-1372: Kobe University Macroalgal Culture Collection (KU-MACC)) was used.

1. Preparation of algae

homogenized and filtered through nylon mesh #263 µm to give the bioassay.

2. Culturing of algae on the test panels

Condition: 15 ℃, 3days with light, 700ml culture solution (50% conc. PESI medium without tris-sodium glycerophosphate), still condition

3. Laboratory bioassay for the efficacy of anti-fouling paints

- The water temperature: 15℃
- The given light intensity by LED lights: 6-18 µmol·m^{-2}·s^{-1}
- Stirr for 15 days

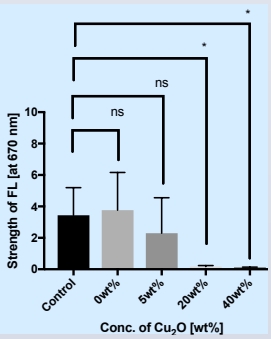
4. Measurement of FL extracted from test panels

Comparison of FL strength on the surface of each panel of experimental and control groups.

Meas. of FL

Figure 4. A flow of the laboratory bioassay using algae.

3. Result



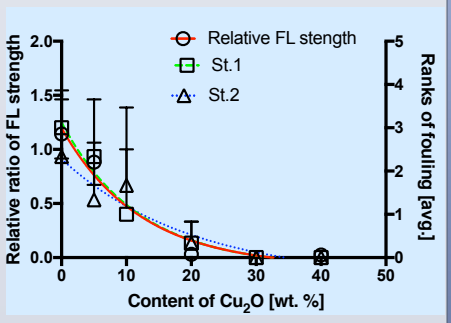


Figure 5. Relative comparison of experiment with control between Cu_2O content and the FL strength (left), relationship between relative FL strength vs ranks of fouling (right) (St.1: Hiroshima, Japan, St.2: Okayama, Japan)

- The fluorescence strength of *E. siliculosus* generally decreased with an increasing of Cu_2O content.
- The critical value of settlement was observed at between 5 and 20 wt. % of Cu_2O.
- Furthermore, the bioassay was validated by comparing results with field experiments, and laboratory results were in good agreement with that of the field.

4. Conclusion

A test protocol using the alga *E. siliculosus* was designed. The following will be further investigated to establish the test protocol for the alga: (1), optimization of its culturing condition; (2), utilization of a flow-through system in the design; and (3), determination of its physiological condition. A draft of the ISO test protocol based on this will be proposed as part of the ISO-21716.

Acknowledgement

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