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Antibacterial and Antialgae Activities and Antifouling Capability of Dithiodipropionamide Derivatives

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Before, the potential property of dithiodipropionamide derivatives were ignored, as the intermediate material of isothiazolones, which were good material in terms of antibacteria, inhibition of algae and antifouling. Here we select three dithiodipropionamide derivatives and studied their capabilities of antibacteria, inhibition of algae and antifouling activity. And taking N,N'-di-butoxymethyl-dithiodipropionamide (BMD) as an example, we obtained that the minimal inhibitory concentration (MIC) of BMD for *Staphylococcus aureus*, *Escherichia coli* and *Fission yeast* were 0.1250, 0.2500 and 0.1250 mg/mL, respectively. The LC_{50} value of BMD were 1.64 and 1.39 mg/L for *Dicrateria zhanjiangensis* at 24 and 48 h correspondingly. Meanwhile we used these three dithiodipropionamide derivatives as antifouling agents in antifouling paint to make test panels, then, immersed test panels in the sea for more than 5 months, resultly, test panels were covered with only a little macroorganism. Thus, the three compounds have potential value as antifoulants.

Key Words: Dithiodipropionamide, Antibacteria, Antialgae, Antifouling.

INTRODUCTION

As a result of the boost in ocean exploring and marine resources developing in recent years, the growth of marine organisms bring dramatic damages to the equipments, such as shipping[1], water pipes[2,3] and drill platform[4] for oil and natural gas and cause huge economic loss[5]. The organisms can slow down the capability and efficiency of these equipments and also cause accidental events and insecurity. In numerous antifouling methods, painting antifouling coating is the most economical, effective and widely used one. Traditional coating[6] used tributyl tin[7] or copper[8] as antifoulants is effective, but caused numerous side-effects to creature. Aggradation of heavy metal leads to teratogenesis of halobios[9], causes environmental pollution[10] and endangers people's lives[11]. They have been total banned globally[12] and then there is vacancy in antifouling coating with high efficiency and low toxicity. Hence, people are in search for new non-toxic, biodegradable, high-effect and environmentally safe antifouling agents.

Isothiazolone derivatives are such environment-friendly antifoulants[13]. In recent years, researchers pay more attention to the use of isothiazolone. Rohm and Haas company explored sea-nine 211[14]. As the active ingredients of sea-nine 211, 4,5-dichloro-2-octyl-4-isothiazol-3-one is famous for low toxicity and friendly to environment and also winned the prize of Presidential Green Chemistry Challenge.

Dithiodipropionamide derivatives are the most important intermediates for synthesizing isothiazolone[15], as the wide application of isothiazolone, dithiodipropionamide derivatives play a crucial role in human lives. For the similar structure of dithiodipropionamide derivatives with isothiazolone, we studied its antibacterial, antialgae activities and application in antifouling painting as antifouling agents.

EXPERIMENTAL

There are three bacteria of *Staphyloccocus aureus* (gram-positive bacteria, ATCC 13565), *Escherichia coli* (gram-negative bacteria, ATCC 12435) and *Fission yeast* (fungi, ATCC 2476) provided by the laboratory of microorganism in the Ocean University of China as well as four algae in terms of *Dicrateria* spp (*Chrysophyta*), *Nitzschia closterium* (*Bacillariophyta*), *Chaetoceros curvisetu* (*Bacillariophyta*), *Platymonas cordiformis* (*Chlorophyta*) supplied by College of Fisheries in ocean university of China. They were cultivated in the biology laboratory and prepared for the experiments. All the chemical reagents were commercially available and treated with standard methods before using. Solvents were dried in rotary evaporator and redistilled. Three tested compounds are N,N'-di-butoxy-methyl-dithiodipropionamide (BMD); N,N'-di-butoxypropyl-dithiodipropionamide (BPD); N,N'-di-phenoxyethoxypropyl-dithiodipropionamide (PEPD), the molecular formula is showed in Fig. 1.

$S-CH_2CH_2CONHCH_2OC_4H_9$
|
$S-CH_2CH_2CONHCH_2OC_4H_9$

BMD

$S-CH_2CH_2CONHC_3H_6O(CH_2)_3CH_3$
|
$S-CH_2CH_2CONHC_3H_6O(CH_2)_3CH_3$

BPD

$S-CH_2CH_2CONHC_3H_6OCH_2CH_2OC_6H_5$
|
$S-CH_2CH_2CONHC_3H_6OCH_2CH_2OC_6H_5$

PEPD

Fig. 1. Structure of three tested compounds

Bacteria inhibition test: Minimal inhibitory concentration (MIC) was determined by test tube double dilution method[16], every sterilization test tube had 2 mL liquid nutrient medium. The positive control group had fungus inoculation but no tested compounds and the negative group was with no fungus inoculation or tested compounds. The initial concentration of the compound was 1 mg/mL and then, doubling dilution in turns, homogeneous mixing. Put 200 μL bacterium solutions into every test tube after a certain time (24 h for *Escherichia coli* and *Staphyloccocus aureus*, 48 h for *Fission yeast*), with definite concentration of the bacterium solutions were 10^4-10^5 cfu/mL (cfu is the formation unit of colony, one cfu is a colony formed on the plate of agar after cultivating). There is turbidity in the positive control group but nothing in the negative group. Finally, the MIC of the three compounds was obtained.

Algal inhibition test: Seawater from the seashore near Qingdao Luxun Park after filtration Sterilization was cooled as solvent of the nutrient solution and the nutrison formula showed in Table-1. The algae (pH 8.0 ± 0.1) were cultured in climate incubator at 21 ± 1 ºC, the photoperiod was 12 h under 3000-4000 Lux, followed by 12 h darkness. We used the same age of algae culture, whose absorbance was between 0.05-0.80, to reduce experimental error and easily compare experiment results.

Algal inhibition evaluation needed 72 h according to toxicity test method for aquatic organism[17]. Firstly, optimal concentration was confirmed by the preliminary experiment and then concentration grads of dithiodipropionamide derivatives were 0, 1.25, 2.50, 5.00, 10.00, 20.00, 40.00 and 80.00 mg/L[18]. Checked absorbency after every certain time, the per cent of death was given according to algal concentration-absorbency linear equation and then χ^2 tests were made to the regression line. Logarithm of LC_{50} value were figured out by the regression equation, which accord with χ^2 test 17 and at last LC_{50} value were attained after concentration conversion.

Board test with antifouling coatings: We studied the capability of antifouling coating according to GB 5370-85, polished armor plates (250 mm × 150 mm × 3 mm) by sand paper, cleaned them by cotton yarn, brushed epoxy primer and antifouling coating on the boards in turn following Table-2, then dried the boards in the air. In the end fastened and immersed testing boards in the sea to a depth of one meter for 173 days.

TABLE-2
COMPOSITIONS OF ANTIFOULING COATINGS (UNIT: g)

Acrylic resin	Cuprous oxide	Pigment	Tested compound	Assistant	Xylene
55	25	17	BMD	2	11
55	25	17	BPD	2	11
55	25	17	PEPD	2	12
55	25	17	0	2	11

RESULTS AND DISCUSSION

MIC of three tested compounds: Following the former study, MIC was the minimal concentration without visible turbidity. So, we could get the MIC of three testing compounds shown in Table-3.

TABLE-3
MIC OF THREE COMPOUNDS (UNIT: mg/mL)

Compound	*Escherichia coli*	*Staphyloccocus aureus*	*Fission yeast*
BMD	0.250 0	0.125 0	0.125 0
BPD	0.250 0	0.062 5	0.125 0
PEPD	0.125 0	0.062 5	0.125 0

From Table-3, it is concluded that three testing compounds were effective to the three bacteria. All of them showed the same effect to *Fission yeast*, BPD and PEPD showed good inhibition to *Staphyloccocus aureus* and better than BMD, PEPD was better than BMD and BPD to *Escherichia coli*. It is found that PEPD was better than BMD and BPD to the three bacteria, just because its phenyl which brought high effect.

Algal inhibition activities

Influence of dithiodipropionamide derivatives with Algae's growth: The concentration of testing compounds was 40 mg/L, according to preliminary experiment. And following this concentration, we could get the influence trend to the growth of algae in 72 h (Figs. 2-5).

From the four figures above, we could see clearly BMD have the best effect on four algae, BPD quickened the growth of *Platymonas cordiformis* and *Nitzschia closterium* at the beginning, but inhibited its growth after about 25 h. N,N'-Diphenoxyethoxypropyl-dithiodipropionamide promoted the

TABLE-1
NUTRIENT SOLUTION FORMULA (w/v)

	NH_4NO_3 (%)	KH_2PO_4 (%)	Carbamide (%)	Ferric citrate (%)	Na_2SiO_4 (%)	V_{B1}, V_{B12}
Platymonas cordiformis	4	0.4	1.8	0.045	0	0
Bacillariophyta	4	0.4	1.8	0.045	2	0
Dicrateria spp	6	0.4	1.8	0.045	0	1.0 mL/1000 mL 0.5 mL/1000 mL

Note: nutrient solution/seawater = 1/1000.

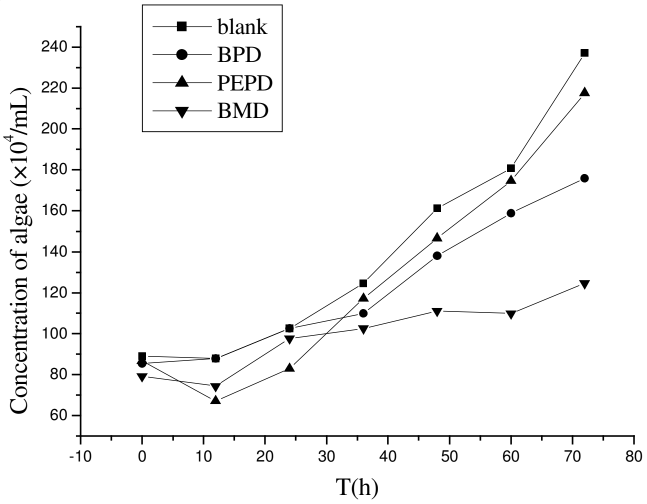


Fig. 2. Effect on *Dicrateria* spp

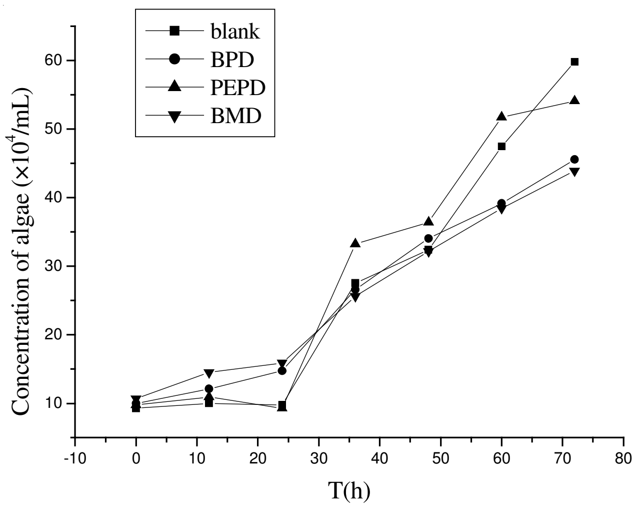


Fig. 5. Effect on *Platymonas cordiformis*

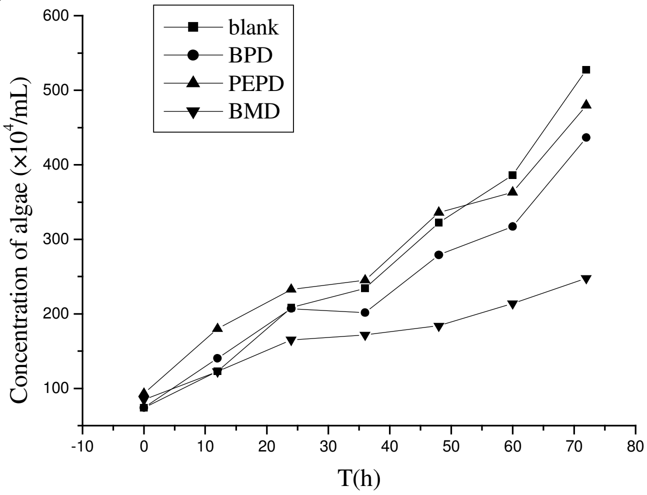


Fig. 3. Effect on *Nitzschia closterium*

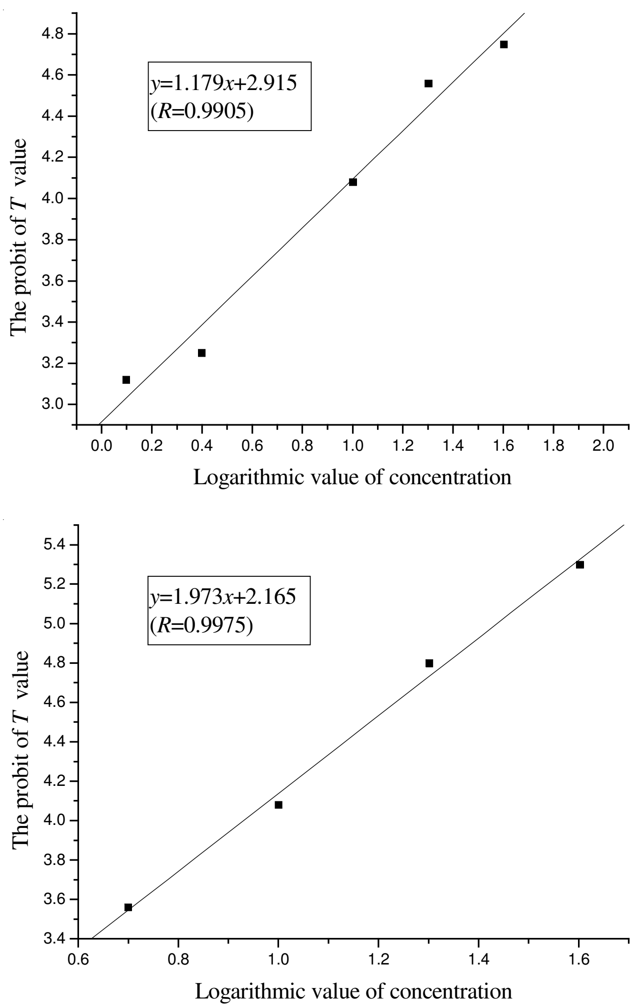


Fig. 6. Linearity of the probit of T value and concentration logarithmic value of BPD after 24 and 48 h exposure

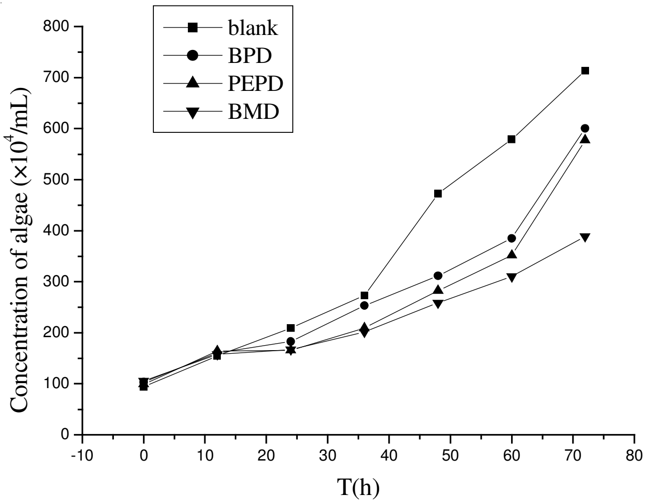


Fig. 4. Effect on *Chaetoceros curvisetus*

growth of *Platymonas cordiformis* and *Nitzschia closterium* more or less. And BMD only promoted the growth of *Nitzschia closterium* in 25 h, subsequently, better inhibited. On the whole, all of them are very effective to *Dicrateria* spp and *Chaetoceros curvisetus*.

Test of LC$_{50}$: Take *Dicrateria* spp for example, the linear equations of the probit of T value and concentration logarithmic value of three tested compounds after 24 and 48 h were presented in Figs. 6-8.

Test of χ^2: χ^2 of all the linearity formulae were tested and compared with $\chi^2_{0.05}$, take BMD with *Dicrateria* spp after 24 h for example and χ^2 was calculated as follows:

From Table-4, we found $\chi^2 < \chi^2_{0.05}$ to BMD in 24 h and meanwhile we could find all the three testing compounds to

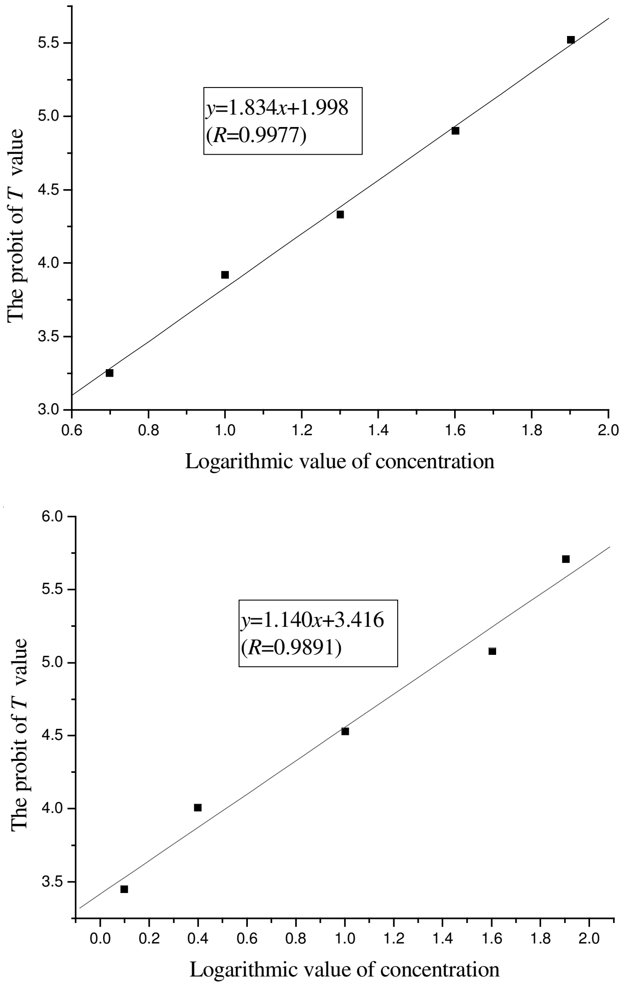


Fig. 7. Linearity of the probit of T value and concentration logarithmic value of BMD after 24 and 48 h exposure

Dicrateria spp: BMD, $\chi^2_{(48\ h)} = 5.48$, BPD, $\chi^2_{(24\ h)} = 4.35$ and $\chi^2_{(48\ h)} = 4.08$, PEPD, $\chi^2_{(24\ h)} = 2.87$ and $\chi^2_{(48\ h)} = 3.67$. All the χ^2 were less than $\chi^2_{0.05}$, therefore, the authenticity of all the tropics was confirmed. LC_{50} were calculated according to the linearity formulae of the probit of T value and concentration logarithmic value (Table-5).

TABLE-5
LC_{50} OF THREE TESTED COMPOUNDS (mg/L)

Tested compound	24 (h)	48 (h)
BPD	1.77	1.44
BMD	1.64	1.39
PEPD	2.10	1.55

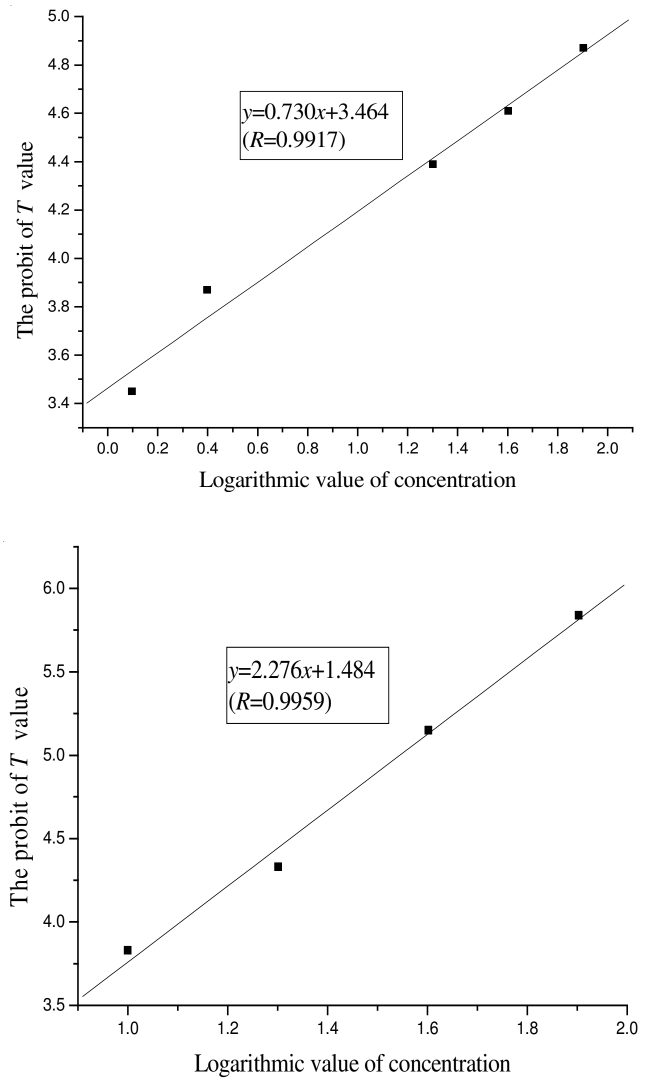


Fig. 8. Linearity of the probit of T value and concentration logarithmic value of PEPD after 24 and 48 h exposure

All the three compounds demonstrated good inhibited activity to *Dicrateria* spp, BMD was better than BPD and PEPD, BMD had the shorted chain which made it better in water-solubility, but that PEPD had the long chain of which decreased its control capability with *Dicrateria* spp. The result from Table-5 was coherent with the result showed in Fig. 2. LC_{50} value for 24 h was less than LC_{50} value for 48 h to every compound, which showed that the toxicity of every algae was enhanced as time increases.

TABLE-4
COUNTING PROCESS FOR χ^2

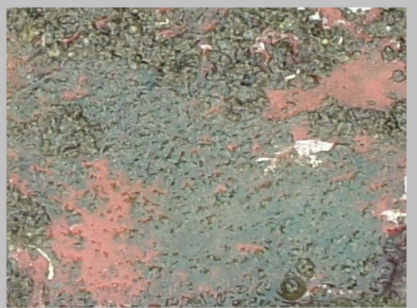
Concentration in logarithmic value (x)	Number of cell (n)	Number of dead cell (r)	Probit of T value (y)	Rate of death (p) (%)	Number of death (np)	$\frac{(r-np)^2}{np(1-p)}$
0.6990	160.02	6.12	3.28	4	6.40	0.0128
1.0000	160.02	24.47	3.83	12	19.20	1.6421
1.3010	160.02	42.83	4.38	27	43.20	0.0045
1.6021	160.02	77.09	4.94	48	76.81	0.0020
1.9031	160.02	118.70	5.49	69	110.4	2.0060
χ^2			3.6673			
$\chi^2_{0.05}$			7.8200			

x: Concentration in logarithmic value, n: total number of cell, r: number of dead cell, y: expected dead probit, p: expected dead rate.

Result of test board in the sea: Being obvious from the studies of antibacterial and antialgae capability, three testing compounds had good inhibition properties, then we put them into antifouling coating as antifoulants and the experiment was conducted in the sea for 173 days (Table-6).

From Table-6, there was no silt or algae for 66 days in the sea on the test boards with antifouling agents, especially board 3. In contrast, the blank board was covered with numerous bacterial slime and some green algae; when immersed in the sea for more than four months, we could see board 2 have the best antifouling capability. There's only a little bacterial slime, silt and *Balanus glandula* on the edge of the board. Board 1 had less bacterial slime but more *Balanus glandula* than board 2. Board 3 was worse than board 1 and 2 but much better than the blank board, which had much macrofouling organisms and algae all over the board; after 173 days, the half paint of the blank board fell off, because that the paint could not bear the weight of macrofouling organisms and the other half was covered

TABLE-6
TEST BOARD IN THE SEA

No.	Time of immerging in the sea (d)		
	66	130	173
1			
2			
3			
Blank			

with plenty of macrofouling organisms and some plankton. Board 2 showed the best antifouling capability, only a little bacterial slime and macrofouling organisms appeared. Board 1 and board 3 had a little paint broken off and other parts were covered with some macrofouling organisms. From the three formulas, antifouling coating with dithiodipropionamide illustrated good antifouling capability against the algae.

Conclusion

In conclusion, all the three testing compounds had good inhibition to some typical bacteria and algae. Especially BMD showed the best inhibition property. It has the shortest alkyl chain which enhanced its water solubility. PEPD was better than BPD, for conjugate benzene ring of PEPD adding its effects. Antifouling coating with three compounds revealed extremely high antifouling capability and lasted for a long time. These means can also be applied to further studies in the field of antifouling technology and the employment of dithiodipropionamide derivatives promote the development of environmentally friendly antifoulants.

REFERENCES

1. Ch.V. Prabhakar and L.M. Rao, *Pollut. Res.*, **19**, 271 (2000).
2. I. Yasuyuki and U. Kazuya, Antifouling Technology for Seawater Intake Pipes of OTEC Using Ozonation, Proceedings of the International Offshore and Polar Engineering Conference, Vol. 16, pp. 337-342 (2006).
3. H.J. Lee, D.G. Han, S.H. Lee, J.W. Yoo, S.H. Baek and E.K. Lee, *Korean J. Chem. Eng.*, **15**, 71 (1998).
4. G.S. Lewbel, R.L. Howard and B.J. Gallaway, *Mar. Environ. Res.*, **21**, 199 (1987).
5. H.G. Xu, H. Ding, M.Y. Li, S. Qiang, J.Y. Guo, Z.M. Han, Z.G. Huang, H.Y. Sun, S.P. He, H.R. Wu and F.H. Wan, *Biol. Invasions*, **8**, 1495 (2006).
6. D.M. Yebra, S. Kiil and D.J. Kim, *Prog. Org. Coatings*, **50**, 75 (2004).
7. P.W. Ball, *Aquaculture*, **65**, 227 (1987).
8. M.C. Finnegan, S. Pittman and M.E. DeLorenzo, *Arch. Environ. Contam. Toxicol.*, **56**, 85 (2009).
9. T. Kiyoshi, *Cell*, **24**, 357 (1992).
10. D. Haynes and D. Loong, *Aust. Environ. Pollut.*, **120**, 391 (2002).
11. M. Hisao, W. Masatomo and W. Keita, *Nippon Ishikai Zasshi*, **121**, 691 (1999).
12. S.M. Evans, A.C. Birchenough and M.S. Brancato, *Marine Pollut. Bull.*, **40**, 204 (2000).
13. A.H. Jacobson and G.L. Willingham, *Sci. Total Environ.*, **258**, 103 (2000).
14. H. Harino, Y. Mori, Y. Yamaguchi, K. Shibata and T. Senda, *Arch. Environ. Contam. Toxicol.*, **48**, 303 (2005).
15. A.J. Lomant, *J. Mol. Biol.*, **104**, 243 (1976).
16. I.A. Muraina, J. Picard and J.N. Eloff, *Phytomedicine*, **16**, 262 (2009).
17. Y.X. Zhou and Z.S. Zhang, Methods of Toxicity Test to Aquatic Organisms, Agriculture Press, Beijing, China, pp. 109-156 (1989).
18. M. Isidori, M. Lavorgna, A. Nardelli, L. Pascarella and A. Parrella, *Sci. Total Environ.*, **346**, 87 (2005).

CARBON ATOM PIONEERS SHARE NOBEL CHEMISTRY PRIZE-2010

Trio separately made outstanding contributions in organic chemistry hailed as 'great art in a test tube'

Stockholm (AFP): Three scientists shared the 2010 Nobel Prize for Chemistry for forging a toolkit to manipulate carbon atoms, paving the way for new drugs to fight cancer and for revolutionary plastics.

Richard Heck of the United States and Eiichi Negishi and Akira Suzuki of Japan were hailed for producing 'great art in a test tube'.

The trio separately made outstanding contributions in organic chemistry, a field whose basis is carbon, one of the essential elements of life and also of innumerable industrial synthetics.

The Nobel has been awarded on four previous occasions for break-throughs in organic chemistry-in 1912, 1950, 1979 and 2005.