PREPARATION, CHARACTERIZATIONANDBIOCIDAL EFFICIENCYOF A QUATERNARY AMMONIUM SALT

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Abstract

A quaternary ammonium salts (QAS) was synthesized from trialkylamine reacting with a halocarbon compound. This QAS was characterized by analytical methods of precipitation and spectroscopic determinations. QAScan damage marine life by killing one-celled plants to destroy their growth systems, and the prepared QAS therefore was used in biocidal experiments for examining their biocidal efficiency towards several bacteria and marine planktonic algas. When the concentrations of the prepared QAS reached at 3.2 and 50 ppm, growth system of the selected bacteria and alga were obviously inhibited respectively.

Keywords

Quaternary ammonium salt; synthesis; characterization; biocidal efficiency.

1. INTRODUCTION

Quaternary ammonium salts (QAS) with alternative alkyls were widely applied as biocides in our daily life and comprehensive improvement of water treatment system for industrial purposes. The cooling systems with directly discharged sea water were extensively adopted by power plants in coastal areas. Along with temperature rising, biological growth was accelerated and as a result, the accumulation of the prolific bacteria in sludge, the algae and shells on surfaces of the cooling systems reduced efficiency of thermal exchange. Oxidative biocides with chlorine were once widely used but have now been superseded by QASs, and novel 'environmentally friendly' biocides with multi-functions have been widely developed and their preparation and application have become more attractive research area.[1-5]Shells are sensitive to toxic or harmful substances, such as strong oxidizing chlorine biocides. Only by closing their valves, they can make safe and protect themselves from damage of chlorine biocides. Beside of this default, chlorine biocides are pollutants for marine environment. [6] But non-oxidative biocides, including QASs with biologically-inspired deceptive behavior, can induce living organisms to contact and accept them, and furthermore to trap, kill and remove living organisms from the surfaces of cooling system for getting high cooling efficiency.[7] In this work a QAS was prepared and characterized, and its biocidal efficiencies were also examined.

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2. EXPERIMENTAL

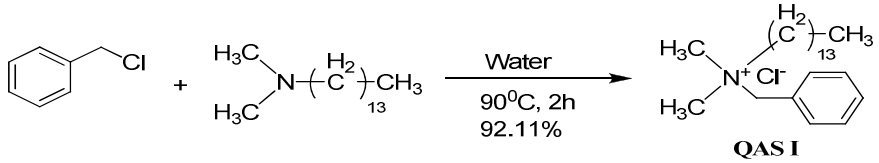
2.1. Measurements

H-NMR spectra were recorded with a Varian Mercury-Plus 300FT-NMR (300 MHz) spectrometer in chloroform-d with tetramethylsilane(Me_4Si) as an internal standard. Chemical shifts (δ) andcoupling constants (J) are given in parts per million and hertzrespectively. Infrared spectroscopy (IR) determinations were performedusing an Avatar 370 FT-IR spectrometer (Thermo Nicolet). Theultraviolet-visible (UV-Vis) spectra were measured by a ShimadzuUV-2450 spectrophotometer, using quartz substrates. Element analyses were carried outin air on a PerkineElmer 240 C elemental analyzer. A Shimadzu LC-20A liquid chromatography was employed for sample's analysis [with C20 column and ultraviolet detector (254 nm), mobile phase was $CH_3CN + H_2O$ (v/v = 3:7)].

2.2. Preparation and analysis for QAS

According to literature method, [8-10] a QAS was designed and prepared. The detail was described as Scheme 1. 92.11% of theoretical QAS I yield was observed experimentally, and it was separated for quantitativeanalysis by perchlorate precipitation method in aqueous solutions. ^{1}H-NMR(300 MHz, $CDCl_3$) δ/ppm: 0.853(t, 3H, -CH_3), 1.243[s, 24H, -$(CH_2)_{12}$-], 1.769(m, 2H, -CH_2-), 2.924(s, 6H, -CH_3), 3.211(t, 2H, N^+-CH_2-), 4.478(s, 2H, benzyl), 7.508(s, 5H, phenyl); IR (KBr)/cm^{-1}: 2920, 2850, 731 and 702(υ_{C-H}), 1631, 1491($\upsilon_{C=C}$, phenyl), 1469(δ_{C-H}, methyl), 1080(υ_{C-N}).

Scheme 1



QAS I

2.3. Antimicrobial activity assay

The prepared QAS I and a commercial product with QAS I were tested against bacterialstrains: Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923). Tests were performed by the standard brothdilution technique [11] with inoculums of approximately1×10^{-5}CFU mL^{-1}. Suspensions of tested compounds werediluted in geometric progression and dispensed into the wellsof a micro-plate. Overnight bacterial culture was diluted withMueller-Hinton broth to the proper density and dispensedinto wells. The growth of bacteria in the wells was examinedafter the incubation of micro-plates for 24-36 h at 37°C.The minimum inhibitory concentration (MIC) was taken as alowest concentration of QAS I that inhibited visible growthof bacteria. Three independent measurements were performedto determine each of the MIC values.

Yeast Extract Peptone Dextrose Medium (YPD): The optimal fermentation medium in solid state was obtained as follow: NaCl5.0 g, beef extract 3.0 g, peptone 10.0 g, agar 20.0 g, water 1000 mL and pH 7.2-7.4 (without agar in the liquid fermentation medium).
Their killing or removing algae efficiencies were also evaluated and analyzedby the efficiencies under conditions of different dose of QAS I, which is a current widely used process to remove algae.

3. RESULTS AND DISCUSSION

3.1. Quantitative analysis of QAS in a biocidal product

QAS component in a biocidal product was quantitativelyanalyzed by perchlorate method: The biocidal product containing QAS single component was deposited by adding excess perchloric acid to form quaternary ammonium perchlorate. After filtration, vacuum drying and weight, component of the QAS was calculated. Determination of molecular structure for the QAS was performed by the mentioned instrumental analyses, including HPLC, electronic spectrum, IR and ^{1}H NMR determinations.

3.2. Biocidal efficiencies

The prepared QAS I with different side groups, including two short methyl, one long tetradecyl, and one aromatic benzyl groups, was designed for suitable to kill different bacteria and living organism.

The biocidal efficiency data of the prepared and the biocide with QAS I were listed in Table 1. The data in Table 1 indicated that the minimum inhibitory concentrations (MIC) for both biocides exhibited a relatively strong inhibitory activity towards Escherichia coli (ATCC 25922) and Staphylococcus aureus(ATCC 25923), and their MIC values were between 1.6-3.2 ppm.

Table 1. The biocidal efficiencies of the prepared and the biocide with QAS I*

Bacterial growth /	Biocidal concentration / ppm									
(Yes or No)	50	25	12.5	6.3	3.2	1.6	0.8	0.4	0.2	0.0
E. coli+A	N	N	N	N	N	Y	Y	Y	Y	Y
S. aureus + B	N	N	N	N	N	Y	Y	Y	Y	Y
E. coli+A	N	N	N	N	N	Y	Y	Y	Y	Y
S. aureus + B	N	N	N	N	N	Y	Y	Y	Y	Y

* *E. coli* = Escherichia coli (ATCC 25922); *S. aureus* = Staphylococcus aureus(ATCC 25923); A = biocide with QAS I; B = the prepared QAS I without purification.

3.3. Algaecidal performance

An unicellular green algae of the genus Chlorella, which is easily cultured and often used in studies of photosynthesis and other experiments, was selected for examining the algaecidal performance from the prepared and the biocide with QAS I. Their algaecidal data were listed in Table 2.

Table 2. Algaecidal effects of the prepared and the biocide with QAS I*

Algae growth /	Biocidal concentration / ppm						
(Yes or No)	5.00	10.0	50.0	100	150	250	500
Green algae+A[a]	Y	Y	Y	N	N	N	N
Green algae + B[a]	Y	Y	Y	N	N	N	N
Green algae+A[b]	Y	Y	N	N	N	N	N
Green algae + B[b]	Y	Y	N	N	N	N	N

[a.]Observation data for 12 h; [b.] Observation data for 24 h.

The experimental results showed that QAS Iprovidedalgaecidal performance. Treated with 1.0 mg/L active green algae, after 12 and 24h,whenalgaecidal ratio was over 99%, the concentrations for both the prepared and the biocide with QAS I were 100 ppmand 50 ppm.These concentrations

were much higher than the MIC for inhibition of growth of variety bacteria, especially fungi, in industrial water treatment system.

3. CONCLUSIONS

A quaternary ammonium salt QAS I was synthesized and characterized. Its biocidal efficiencies to inhibit bacterium growth and to kill green algae were investigated. Experimental results indicated that the prepared QAS I and the commercial biocide product with QAS I exhibited the same antimicrobial efficiencies. The MIC for inhibition towards *Escherichia coli* and*Staphylococcus aureus*were between 1.6-3.2 ppm. Meanwhile the simples with 50-100 ppm of the prepared QAS I and the commercial product presented much high efficiency to kill the selected green algae. During biocidal process, QASs with different alkyl and aryl groups are sensitive to kill different bacteria, algae and sea-shells. Therefore a complicated biocide with different QASs should be provided in the practice application. Especially, QAS can destroy the osmosis system of cell, allowing the cell to become turgid and bursting and causing loss of water. This function can also be applied in sludge dewatering to improve sewage treatment system. These experiments are undergoing.

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REFERENCES

[1] T. R. Bott, Techniques for reducing the amount of biocide necessary to counteract the effects of biofilm growth in cooling water systems, Appl. Thermal Engineering, 1998, 18, 1059-1066.
[2] M. R. Viera, P. S. Guiamet, M. F. L. de Mele, H. A. Videla, Use of dissolved ozone for controlling planktonic and sessile bacteria in industrial cooling systems, Intern. Biodeter. Biodeg., 1999, 44, 201-207.
[3] G. Sundheim, S. Langsrud, E. Heir, A. L. Holck, Bacterial resistance to disinfectants containing quaternary ammonium compounds, Intern. Biodeter. Biodeg., 1998, 41, 235-239.
[4] J. A. Callow, M. E. Callow, Trends in the development of environmentally friendly fouling-resistant marine coatings, Nature Communications, 2011, 2, 244.
[5] M. J. Ford, L. W. Tetler, J. White and D. Rimmer, Determination of alkyl benzyl and dialkyl dimethyl quaternary ammonium biocides in occupational hygiene and environmental media by liquid chromatography with electrospray ionisation mass spectrometry and tandem mass spectrometry, J. Chromatography A, 2002, 952, 165-172.
[6] L. A. Mayack, R. J. Soracco, E. W. Wilde and Daniel H Pope, Comparative effectiveness of chlorine and chlorine dioxide biocide regimes for biofouling control, Water Research, 1984, 18, 593-599.
[7] J. A. Callow, and M. E. Callow, Trends in the development of environmentally friendly fouling-resistant marine coatings, Nat. Commun., 2011, (2), 244.
[8] M. Fan, C. Luo, X. Wei, and B. Ni, Synthesis of biodegradable ester-containing quaternary ammonium salt by a novel route, J. Taiwan Institute of Chem. Engineering, 2013, 44, 202-204.
[9] Z. Jia, D. Shen, W. Xu, Synthesis and antibacterial activities of quaternary ammonium salt of chitosan, Carbohydrate Research, 2001, 333, 1-6.
[10] W. Wang, Z. Bai, F. Zhang, C. Wang, Y. Yuan, J. Shao, Synthesis and biological activity evaluation of emodin quaternary ammonium salt derivatives as potential anticancer agents, European Journal of Medicinal Chemistry, 2012, 56, 320-331.
[11] D. R. Stalons, and C. Thornsberry, Broth-Dilution Method for Determining the Antibiotic Susceptibility of Anaerobic Bacteria, Antimicrobial Agents and Chemotherapy, Jan. 1975, 15-21.

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