Preparation of carboxymethyl-quaternized oligochitosan and its scale inhibition and antibacterial activity

Hui-xin Zhang, Dong-xue Sun, Yu-chao Zhu, Ting-ru Yang, Xiu-hong Jin and Feng Wang

ABSTRACT

Carboxymethyl-quaternized oligochitosan (CMQAOC) was prepared through carboxymethylation and N-quaternization of oligochitosan (OC) which was obtained from chitosan degradation. The structure was confirmed by Fourier transform infrared spectroscopy (FT-IR), 1H-NMR, and X-ray diffraction (XRD) analysis. The performances for both scale and microbial inhibition of CMQAOC were evaluated by static test and culture flask method, respectively. The results showed that the scale inhibition rate for calcium carbonate and calcium sulfate scale were both beyond 80% with a CMQAOC dosage of 20 mg/L. With a degree of substitution for quaternary ammonia of up to 0.74, the killing rate for saprophytic bacteria and sulfate-reducting bacteria was 98.9 and 100%, respectively. The ratio of biochemical oxygen demand/chemical oxygen demand (BOD5/COD) of CMQAOC was 0.45, showing that CMQAOC is as biodegradable as OC.

Key words | biodegradation, carboxymethyl-quaternized oligochitosan, microbial inhibition, scale inhibition

INTRODUCTION

As the deacetylated product of chitin, chitosan is a unique cationic alkaline polysaccharide with a stable structure with an average molecular weight ranging from thousands to millions in nature (Dutta et al. 2002; Kurita et al. 2010). In chitosan macromolecular chains there are a large number of -NH2 and -OH groups that can act as reactive sites for versatile chemical modification. The introduction of hydrophilic groups such as acyl, carboxyl, and quaternary ammonium can improve its poor solubility in water (Mourya & Inamdar 2008; Yeul & Rayalu 2015). As a result, chitosan and its derivatives are widely used already in foods, textile industry, chemical industry, agriculture practices, bio-engineering, etc. (Goosen 1997; Jiang 2003). Scaling, corrosion, and the generation of sludge and fouling from bacteria, fungi, and algae are three kinds of major damage in various industrial water systems and practices such as water injection in oil fields, multi-stage flash desalination, brackish water desalination, reverse osmosis operation, and sea water desalination (Liu et al. 2013). Nowadays, resource-saving and environment-friendly water chemicals are taken into consideration. Therefore, chitosan and its derivatives have received much more attention in recent decades due to it being an inexpensive, abundant resource, and considered green due to its biodegradability biocompatibility and biological activity (Rinaudo 2006; Kean & Thanou 2010). Carboxymethyl-chitosan is an amphiprotic ether derivative, which contains active hydroxyl (-OH), carboxyl (-COOH), and amine (-NH2) groups in the molecule, making it possible to prevent scale formation through chelation and dispersion of metal ions, or via lattice distortion of scaling ingredients (Zhang et al. 2000; Liu et al. 2001; Chen & Park 2003; Wang & Wang 2008). The introduction of N-trimethylated quaternary ammonium salt group would greatly weaken the hydrogen bonds between the chitosan molecule chains, and improve water-solubility, antibacterial activity and the moisture absorption and retention.

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capabilities of chitosan (Deng & Chen 2009; Yu et al. 2011; Lin et al. 2012). Some reports have suggested that quaternary ammonium salts of chitosan with appropriate degrees of quaternization are soluble in neutral or even slightly alkaline aqueous solvents; they also show markedly improved antimicrobial activities as compared to unmodified chitosan in pH-regulated environments (Jintapattanakit et al. 2008; Wu et al. 2011; Kucukgulmez et al. 2012).

In an attempt to obtain a novel green multi-function water chemical, this paper reports the preparation of amphiprotic chitosan derivatives in which both carboxymethyl group and quaternary ammonium group are introduced together onto chitosan molecular chains. The raw chitosan was first degraded to oligochitosan (OC) with much better solubility and reactivities and then modified through the reaction with chloroacetic acid and 2,3-epoxypropyl trimethyl ammonium chloride (Figure 1). Carboxymethyl-quaternized oligochitosan (CMQAOC) inhibition to both scaling and bacteria growth, and its biodegradability were evaluated in detail.

**MATERIALS AND METHODS**

**Materials**

Chitosan (degree of deacetylation >95%, viscosity average molecular weight of 350 kDa) was purchased from Jinan Haidebei Marine Bioengineering Co. Ltd (Shandong, China). The KBC series of bacterial test bottles were purchased from Zhengzhou Water Testing Technology Co. Ltd (Henan, China). All other reagents were of analytical reagent grade and were used without further purification.

**Preparation of oligochitosan**

Chitosan (3.00 g) was dissolved in a 1% w/v acetic acid solution (40 mL) with stirring for 30 min. A 5% w/w hydrogen peroxide solution (30.00 mL), was pipetted into the above viscous liquor, and stirred for another 10 min. The resulting homogeneous solution was then irradiated with an anodic current of 70 mA at 70 °C for 6 min in a microwave (MW) reactor. The resultant liquor was cooled to ambient temperature, neutralized with a 5% w/w sodium hydroxide solution and filtered through a funnel. The filtrate obtained was precipitated by pouring into absolute ethanol (60 mL). The precipitate was filtered, washed with ethanol (60 mL) three times, then dried overnight under vacuum at 40 °C.

The viscosity average molecular weight ($M_v$) of OC was determined by a one-point method, the solvent was a 0.1 mol/L CH$_3$COOH/0.2 mol/L NaCl solution. The efflux times of a series of chitosan solutions and blank solvent were measured in triplicate using an Ubbelohde capillary viscometer in a constant-temperature water bath at 25 °C. The values were recorded as $t$ and $t_0$ in seconds and used to calculate the intrinsic viscosity $\eta$. Then, $M_v$ of OC was calculated based on the Mark–Houwink equation (Wang et al. 1991) as
in Equation (1). The polydispersity \((D)\) was calculated by Equation (2) (Chin et al. 1994; Lynd et al. 2008)

\[
\eta = \frac{[4\ln (t/t_0)]}{t/t_0 - 1 + 3 K M_\nu^c} \quad \text{(1)}
\]

where \(K\) and \(\alpha\) are the constants based on the nature of the polymer and solvent material as well as on temperature, and here have values \(1.81 \times 10^{-3} \text{ cm}^3/\text{g}\) and 0.93, respectively (Roberts & Domszy 1982)

\[
D = \frac{M_w}{M_n} \quad \text{(2)}
\]

where \(M_w\) is weight average molecular weight, \(M_n\) is number average e mass. Results show that \(M_n\) of OC was \(~6 \text{ kDa}\) and polydispersity \((D)\) was 1.74, and this suggests the OC having a narrow molecular-weight distribution.

**Preparation of CMQAOC**

OC (3.00 g), suspended in isopropyl alcohol (20 mL), was mixed with sodium hydroxide solution (20\%, 10 mL) in a three-necked flask under stirring at room temperature overnight. Chloroacetic acid (6.00 g in 20 mL isopropyl alcohol) was slowly added into the three-necked flask with stirring. The content in the three-necked flask was heated to 60 °C in the water bath and allowed to react for 4 hours (Cerrutti et al. 2013). Then, the resultant liquor was adjusted to \(\text{pH} = 8-9\) using glacial acetic acid. The temperature of the water bath was raised to 80 °C. A certain amount of glycidyl trimethyl ammonium chloride (GTMAC) dissolved in distilled water (20 mL) was dropped into the three-necked flask through a constant pressure dropping funnel in three portions in 2-hourly intervals. The reaction was carried out totally for about 10 hours. The clear and yellowish reaction solution was poured into cold acetone (100 mL) under stirring and left in a refrigerator (5 °C) overnight. The settled solid was filtered and washed with an acetone–ethanol mixture (4:1, v/v) and acetone, and then dried under a vacuum at 50 °C to obtain a pale yellow product.

The degree of substitution of carboxymethyl (DSC) for CMQAOC was determined by using potentiometric titration (Wang et al. 2010). The degree of substitution of quaternary ammonium group (DSQ) for CMQAOC was determined by using AgNO₃ titration (Li et al. 2004).

**Characterization of OC and CMQAOC**

OC and CMQAOC were subjected to Fourier transform infrared spectroscopy (FT-IR), \(^1\)H-NMR, and X-ray diffraction (XRD) study. The IR spectra were collected at a resolution of 4.0 cm\(^{-1}\) over 4,000–400 cm\(^{-1}\) using a Bruker Vensor 27 FT-IR spectrophotometer in KBr pellets against dry air background with DTGS detector. The \(^1\)H-NMR spectra was acquired at 25 °C in D₂O on a Bruker AVANCE 400 NMR spectrometer. XRD diffraction patterns were recorded in a Philips X’Pert MPD X-Ray Diffractometer using Cu Kα radiation at 0.154 nm over a 20 range of 5–40°.

**Antiscalin evaluation for CMQAOC**

The inhibition of CMQAOC to calcium carbonate scaling and calcium sulfate scaling were evaluated by individual static antiscalin testing from GB/T 16632-2008 (China). Distilled water, CaCl₂ solution, NaHCO₃ or Na₂SO₄ solution, and the potential scale inhibitor were added into 500 mL conical flasks individually against dosages. A borax buffer solution was employed to adjust to pH 9.0. These mixture solutions were regulated to a fixed volume with distilled water for each set of experiments. Test samples free or with OC or its derivative (of different dosing) in the conical flasks were then placed into the water bath at 80 °C for 10 hours. After completion, the aliquots were rapidly cooled to room temperature, filtered and immediately analyzed for residual soluble calcium by EDTA titration employing calcine as an indicator. The scale inhibition rate \(\eta\) is calculated according to Equation (3)

\[
\eta = \frac{C_1 - C_2}{C_0 - C_2} \times 100\% \quad \text{(3)}
\]

where \(C_0\) is initial soluble calcium concentration before incubation in mg/L, \(C_1\) is the final soluble calcium concentration for the inhibited sample after incubation in mg/L, \(C_2\) is the final soluble calcium concentration in the blank test after incubation in mg/L.

The effect on the scale inhibition rate was considered by different Ca\(^{2+}\) concentrations and inhibitor dosages. The concentration of HCO₃⁻ and SO₄²⁻ were regulated to keep the mole ratio of HCO₃⁻ or SO₄²⁻ to Ca\(^{2+}\) at 2:1 or 1:1 in all test samples for scaling inhibition to CaCO₃ or CaSO₄.
SEM images of scaling product from static evaluation

Scanning electron microscopy (SEM) was used to investigate the morphology of the scaling products from the static antiscaling tests. The images were observed using an S-4800 SEM (Hitachi, Japan) at 3.0 kV.

Extinction dilution evaluation for CMQAOC antimicrobial activity

The extinction dilution method was employed to evaluate the effect of CMQAOC on the generation of heterotrophic bacteria (TGB) and sulfate-reducing bacteria (SRB), and the numbers of bacteria were counted with the most probable number method (De Oliveria et al. 2011). Test strains, originated from the recirculated cooling water system in a chemical plant, were trained in the culture medium and diluted with sterile standard water at a ratio of 1:50 before application. CMQAOC was added into the dilution against dosages and maintained contact with the bacteria strains for 4 hours. The culture medium was followed at 35 °C for 7 days. The observation of precipitation, turbidity or a yellow color (for TGB) or black color (for SRB) indicated the growth of heterotrophic bacteria. The killing ratio \( X \) is calculated as follows in Equation (4)

\[
X = \frac{A - B}{A} \times 100\%
\]  

(4)

where \( A \) is the number of bacteria for the blank test without biocide in numbers per mL; \( B \) is the survival number of bacteria with an addition of biocide in numbers per mL.

Determination of biodegradation rate (BOD\(_5\)/COD)

The sample solution was prepared by dissolving OC or CMQAOC (1.00 g) in water (1 L). Chemical oxygen demand (COD) was determined with the sealing method (Ping 2004). The sample solution (1.0 g/L, 2.50 mL) was placed into a 50 mL colorimetric tube and mixed with some digestive solution (0.250 mol/L 1/6K\(_2\)Cr\(_2\)O\(_7\) and 33.3 g/L HgSO\(_4\), 2.50 mL) and a catalyst solution (8.8 g Ag\(_2\)SO\(_4\) – dissolved in 1 L concentrated H\(_2\)SO\(_4\), 3.50 mL). The colorimetric tube was taken to the incubator and digested at 150 °C for 2 hours. The final solution was titrated with 0.1 mol/L (NH\(_4\))\(_2\)Fe(SO\(_4\))\(_2\)·6H\(_2\)O standard solution, and meanwhile the test was compared with the blank test. The COD is calculated as follows in Equation (5)

\[
\text{COD}(\text{O}_2, \text{mg/L}) = \frac{(V_0 - V_1) \times C \times 8 \times 1000}{V}
\]  

(5)

where \( V_1 \) is the volume of (NH\(_4\))\(_2\)Fe(SO\(_4\))\(_2\) standard solution for sample solution in mL; \( V_0 \) is the volume of (NH\(_4\))\(_2\)Fe(SO\(_4\))\(_2\) standard solution for blank test in mL; \( C \) is the concentration of (NH\(_4\))\(_2\)Fe(SO\(_4\))\(_2\) standard solution in mol/L, 8 is the molar mass of oxygen in g/mol, and \( V \) is the volume of sample solution in mL.

Biological oxygen demand (BOD) was determined for 5 days of cultivation at a constant temperature of 20 °C (Seo et al. 2007). The aerated and inoculated dilution water (CaCl\(_2\), FeCl\(_3\), MgSO\(_4\), KH\(_2\)PO\(_4\), K\(_2\)HPO\(_4\), Na\(_2\)HPO\(_4\), and NH\(_4\)Cl) was drawn into the dissolved oxygen flask (250 mL) by siphon firstly, and then was fed with 2.50 mL of the sample solution. The dissolved oxygen flask was filled with and then sealed with dilution water. The dilution method consists of measuring dissolved oxygen (DO) of the sample at the beginning and after the incubation period; and meanwhile the test was compared with the blank test. The BOD is calculated as follows in Equation (6)

\[
\text{BOD}_5(\text{O}_2, \text{mg/L}) = \frac{(C_1 - C_2) - (B_1 - B_2) \times f_1}{f_2}
\]  

(6)

where \( C_1 \) is the DO of sample solution before incubation period in mg-O\(_2\)/L, \( C_2 \) is the DO of sample solution after incubation period of 5 days in mg-O\(_2\)/L, \( B_1 \) is the DO of dilution water before incubation period in mg-O\(_2\)/L, \( B_2 \) is the DO of dilution water after incubation period of 5 days in mg-O\(_2\)/L, \( f_1 \) is the proportion of the diluting water in the culture solution in percent, \( f_2 \) is the proportion of sample solution in the culture solution in percent.

The biodegradation rate is equal to the ratio of BOD\(_5\)/COD. When the ratio was greater than 0.45, it is regarded as easily biodegradable; when the ratio was greater than 0.30, it is regarded as biodegradable; when the ratio was less than 0.30, it is regarded as non-biodegradable (Chamarro et al. 2001).
RESULTS AND DISCUSSION

Characterization of OC and CMQAOC: FT-IR analysis

The FT-IR spectra of raw chitosan, OC, and CMQAOC is presented in Figure 2. The 3,400 cm\(^{-1}\) wide peak is formed from the overlap of the -NH\(_2\) and -OH stretching vibrations. The absorption at 1,655 cm\(^{-1}\) in the spectrum of chitosan can be assigned to amide I. The fact that bending vibration of -NH\(_2\) in OC is strengthened at 1,598 cm\(^{-1}\) indicates degradation disrupts the hydrogen bonding among the chitosan molecular chains. The C-O stretching vibrations for the primary hydroxyl group and secondary hydroxyl group appear at 1,030 and 1,080 cm\(^{-1}\). These peaks take similar locations and shapes for chitosan and OC. Meanwhile, OC still presents two characteristic absorption peaks for the \(\beta\)-1,4 glycosidic bond at 1,151 and 894 cm\(^{-1}\). This suggests that the degradation process did not change the basic structure of chitosan chains. Two strong peaks at 1,601 and 1,415 cm\(^{-1}\) in the CMQAOC spectrum are observed due to the asymmetrical and symmetrical stretching of COO\(^{-}\) (Liu et al. 2001). In the spectrum, the C-O stretching band at 1,030 cm\(^{-1}\), corresponding to the primary hydroxyl group, disappears – verifying a high carboxymethylation of OH-6. The characteristic peak of the secondary hydroxyl group at 1,080 cm\(^{-1}\) changes little, but the 1,200 cm\(^{-1}\) band has been enhanced and presents new peaks near 570 cm\(^{-1}\). This indicates that carboxymethylation mainly occurred on the hydroxyl group. The absorption bands at 1,375 and 1,406 cm\(^{-1}\) originate respectively from -CH\(_3\) and -CH\(_2\) stretching vibration on quaternary ammonium moieties (Mohamed et al. 2013), which indicates that the quaternary ammonium groups were successfully grafted.

Characterization of OC and CMQAOC: \(^1\)H-NMR analysis

Figure 3 shows the \(^1\)H-NMR spectrum of CMQAOC in D\(_2\)O solvent. The peak at \(\delta\) 2.00 ppm is assigned to the proton of residual CH\(_3\) acetyl, \(\delta\) 2.65 ppm is the CH\(_2\) absorption peak of C2-N-carboxymethyl functional group. \(\delta\) 3.70–3.90 ppm and \(\delta\) 4.41 ppm are the CH\(_2\) absorption peaks of C3-O-carboxymethyl group and C6-O-carboxymethyl group. The most intense signal at \(\delta\) 3.16 ppm (except for the solvent peak at about 4.7 ppm) is attributed to the protons of the methyl groups of the quaternary ammonium salt. The chemical shifts for the chitosan backbone hydrogens are between 3.50 and 4.00 ppm. The peaks at \(\delta\) 4.49, 3.17, 3.57, 3.73, 3.63, and 3.88 ppm are attributed to H-1, H-2, H-3, H-4, H-5, and H-6, respectively. The peak at \(\delta\) 4.28 ppm is attributed to the CH\(_2\) from the carboxymethyl group. The peaks at \(\delta\) 2.21, \(\delta\) 4.14 and \(\delta\) 3.28 ppm were attributed to the H-a, H-b, and H-c, respectively. Similar to the results obtained by Mohamed et al. (2013), \(^1\)H-NMR data demonstrate accurately that the quaternary ammonium moieties were introduced to chitosan chains.

Characterization of OC and CMQAOC: XRD analysis

The X-ray diffraction patterns for chitosan, OC, and CMQAOC are shown in Figure 4 with a copper target and...
a diffraction angle between 5° and 40°. Chitosan has two sharp peaks in the range of 5–40° and the largest peak is located at 21°. OC shows two broad and blunt peaks, which implies a decreasing crystallinity. What can account for this phenomenon is that the crystalline region has been degraded and hydrogen bonding between amino and hydroxyl groups are weakened in OC molecular chains. As bulk functional groups are introduced into OC backbone, the ordered arrangement of chitosan caused by corresponding intermolecular and intramolecular hydrogen bonds are disrupted, which accounts for the lower crystallinity of CMQAOC. Consequently, the water solubility of chitosan is improved, which has a great significance for application in water treatment.

Performances for scaling inhibition

Inhibition performance on CaCO₃ scaling

The inhibition efficiencies of OC and CMQAOC on calcium carbonate scaling were studied against different Ca²⁺ concentrations and the results are presented in Figure 5. The tests were carried out with 20 mg/L of OC or CMQAOC and 732 mg/L of HCO₃⁻ at pH 8. The antiscalant performance of CMQAOC improves significantly compared to OC. The rate decreased slowly with an increasing Ca²⁺ concentration from 240 to 388 mg/L, and decreased rapidly after 550 mg/L. The rate fell by about 20% with a doubling in Ca²⁺ concentration from 240 to 500 mg/L. The rate for CMQAOC was still above 70%, while only being 18% for OC, even with Ca²⁺ concentration of 465 mg/L. This indicates that CMQAOC tolerance to calcium ions is high and stable under high hardness and alkalinity. CMQAOC contains large amounts of carboxyl, amino, and hydroxyl groups. These active groups could bind Ca²⁺ to form polymeric chelates in solution. The crystal lattices are thus distorted and the capability of bridge bonds is lost preventing the calcium carbonate scale from being formed (Kumar et al. 2010).

Figure 6 shows that CMQAOC presented considerably stronger antiscalant activity than that for OC at the same
dosage. Scale inhibition rate keeps steadily increasing with increasing dosage, and finally reaches 90% when the dosage of CMQAOC is greater than 20 mg/L. This demonstrates the excellent inhibition performance of CMQAOC on calcium carbonate scaling. The calcium carbonate scale becomes an amorphous, loose structure with crystal distortion in the presence of CMQAOC – as seen in SEM images – helping scale components to be dispersed or dissolved in water.

**Inhibition performance on CaSO₄ scaling**

The inhibition of OC and CMQAOC to calcium sulfate scaling was determined against different Ca²⁺ concentrations at pH 9 with an addition of 20 mg/L of the scale inhibitor and a mole ratio of 1:1 for SO₄²⁻ to Ca²⁺. The results are shown in Figure 7, and are similar to Figure 5. With an increasing concentration of scale components, the inhibition rate of CMQAOC decreased steadily before Ca²⁺ concentration of up to 2,100 mg/L. After this point, the inhibition rate of CMQAOC declined further, but is still above 60% when the Ca²⁺ concentration is 2,200 mg/L. At this point, the inhibition rate of OC is only 15%. Thus, CMQAOC has a very clear and relatively stable tolerance to CaSO₄ scale components. The reasons for this high scale inhibition rate result from much more abundant -COOH, -OH, and -NH₂ groups existing in CMQAOC. With increasing dosage, more and more polar groups interacted with the surface of the calcium sulfate crystals, changing the charge density of the particle surface. Consequently, the repulsion among these particles is enhanced, leading to precipitation, more open structure and crystalline distortion (Saleah & Basta 2008). As a result, the crystallization rate decreased and the scale inhibition rate increased. In summary, CMQAOC was considered as an excellent inhibitor of CaCO₃ and CaSO₄ scaling.

**SEM observation for scaling products**

The surface morphology of calcium carbonate or calcium sulfate crystals upon addition of the inhibitor was studied through SEM. Figure 9 shows images of scaling crystals, free or in the presence of CMQAOC, as obtained from the static antiscaling evaluation.
Figure 9(a) shows that CaCO₃ crystals obtained in the absence of inhibitors are clear cubic blocks with clear edges and corners, which is typical for calcite (Kumar et al. 2010). These are adherent and tenacious in nature due to their regular and compact structure. In the case of inhibitors being present, CaCO₃ crystals become irregular and rough with a smooth and looser structure (Figure 9(c)). They are therefore less adhesive and could be easily dispersed; similarly for the CaSO₄ crystals, which are shown in Figures 9(b) and 9(d). Thus the crystals in Figures 9(c) and 9(d) are more difficult to attach to the surface of the flask and easier suspended or washed away by a water stream. In fact, it was observed that the scaling products obtained free of inhibitors mostly settled down in the form of hard scale at the bottom of the flask. The scaling products obtained with inhibitor addition mainly settled down on the wall and bottom of the flask in a relatively loose and bulk sludge while some of the material was suspended as a kind of slurry in static experiments.

The morphological study of the scale crystals (in the presence and absence of CMQAOC) suggested that CMQAOC interfered with the formation and the growth of inorganic scale crystals through a chelating ability and acted as a crystal distortion inhibitor. The scale inhibition mechanism of modified chitosan could be assumed as follows (Zhang et al. 2007; Kumar et al. 2010): the complexation and adsorption of CMQAOC are greatly enhanced due to the introduction of carboxylate, hydroxyl, and amino groups, so that metal ions form chelates in solution. The reason for amorphous deformation, loose structure and crystal distortion of scaling precipitation resulted from the addition of chitosan derivatives in which -COO⁻, -OH, -NH₂ and quaternary ammonium salt groups were abundant. They interacted with the surface of calcium carbonate or calcium sulfate crystals and occupied the crystal growth loci, preventing crystal surface diffusion and the positioning of ions or molecules, inhibiting crystal growth. On the other hand, the scaling particles would be surrounded due to adsorption of CMQAOC and the charge
nature and intensity of the particle surface changed. Consequently, the repulsion among these particles becomes enhanced and the crystallization rate decreased. The crystal lattices were distorted and the capability of bridging interactions was lost, so that the scaling components could be stable in a dispersed state in solution.

**Antimicrobial activity of CMQAOC**

In water, quaternary ammonium salts dissociate into large molecular weight organic cations and low molecular weight inorganic anions. The cationic moieties generally have hydrophilic groups centered around nitrogen atoms, so that the quaternary ammonium salt has a strong surface-active role. Bacteria can be viewed as biological colloids in water which usually bear a negative charge. Quaternary ammonium cations kill the bacteria through adsorbing onto the bacterial surface and damaging the cell permeability of the plasma membrane. The hydrophobic groups and hydrophilic groups of quaternary ammonium infiltrate into the bacterial cell membrane lipid and protein layers, respectively (Kim *et al.* 1997), thus the bacterial cell membrane permeability is altered. The quaternary ammonium salt of OC enters into the body of bacteria, interfering with bacterial proteins or lipoproteins, causes protein denaturation, hinders metabolism resulting in bacterial death. It could also be caused by its water-binding capacity and inhibition of various enzymes; in addition, chitosan could inhibit the bacterial development as it absorbs the bacterial nutrients.

The bactericidal effects for TGB and SRB of OC and CMQAOC having various DSQs are presented in Table 1.

The dosage of OC and CMQAOC was 30 mg/L equally. The bactericidal rates for TGB and SRB were respectively 23 and 35% for OC. In experiments, CMQAOC having various DSQ could be obtained by adjusting charge ratio of OC to GTMAC. When charge ratio of OC with GTMAC was 1.00:2.00, CMQAOC could be obtained with a substitution degree of quaternary ammonium of 0.74, and its killing ratio to TGB and SRB were 98.9 and 100%, respectively. CMQAOC exhibited antimicrobial activity superior to OC; this is attributed to the quaternary ammonium groups, and antimicrobial activity improves with the increase of DSQ.

**Biodegradability**

The BOD₅/COD ratio has been employed as biodegradability indicator. When the ratio was greater than 0.45, it may be considered thoroughly biodegradable; when the ratio was greater than 0.30, it implied biodegradable; when the ratio was less than 0.30, it was regarded as non-biodegradable (Chamarro *et al.* 2001). Results in Table 2 show that OC and CMQAOC have a biodegradability ratio (BOD₅/COD) of 0.80 and 0.45, respectively. Therefore, the OC and CMQAOC were both easily biodegradable.

**CONCLUSIONS**

CMQAOC was prepared successfully with OC which was obtained via a MW/H₂O₂ degradation. The chemical structures and physical properties of CMQAOC were characterized by FT-IR, ¹H-NMR, and XRD. The performances and mechanism of CMQAOC for both CaCO₃ and CaSO₄ scaling inhibition were assessed in detail. The results reveal CMQAOC had a good adaptability to the Ca²⁺ in pH 8–9. The inhibition ratio for calcium carbonate scale was more than 70% when the dosage of CMQAOC was 20 mg/L, Ca²⁺ concentration ≤465 mg/L, HCO₃⁻.

<table>
<thead>
<tr>
<th>Charge ratio of OC to GTMAC (mol/mol)</th>
<th>Degree of substitution of quaternary ammonia</th>
<th>Antibacterial rate (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>TGB</td>
</tr>
<tr>
<td>1.00:0.50</td>
<td>0.45</td>
<td>90.2</td>
</tr>
<tr>
<td>1.00:1.00</td>
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</tr>
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<td>1.00:2.00</td>
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<td>98.9</td>
</tr>
<tr>
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<td>98.7</td>
</tr>
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<td>OC</td>
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<table>
<thead>
<tr>
<th>Name</th>
<th>COD  (O₂, mg/L)</th>
<th>BOD₅ (O₂, mg/L)</th>
<th>BOD₅/COD</th>
<th>Biodegradability</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC</td>
<td>787.26</td>
<td>631.08</td>
<td>0.80</td>
<td>Easily</td>
</tr>
<tr>
<td>CMQAOC</td>
<td>429.69</td>
<td>194.69</td>
<td>0.45</td>
<td>Easily</td>
</tr>
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</table>
concentration = 732 mg/L. The inhibition ratio for calcium sulfate scale was 80% higher when dosage of CMQAOC was 20 mg/L, Ca²⁺ concentration ≤ 2,050 mg/L, SO₄²⁻ concentration ≤ 4,920 mg/L, pH = 8. Compared to OC, CMQAOC had an excellent bactericidal activity. When the dosage of CMQAOC (DSQ was 0.74) was 30 mg/L, the bactericidal killing ratios of TGB and SRB were 98.9 and 100%, respectively. The ratios of BOD₅/COD of OC and CMQAOC were determined as 0.80 and 0.45, respectively, both beyond the upper limit value 0.30, which indicated both OC and CMQAOC were biodegradable and ‘green’.

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