J. Chem. Chem. Eng. 1 (2016) 42-47
doi: 10.17265/1934-7375/2016.01.005

Droplet Contact Angle Measurement on Microstructures of Octocoral Sclerites-ESEM Image Analysis

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Abstract: This work explores the methodology for micron-scale water droplet contact angle derivation for the warty surface of octocoral sclerites. The calcite-made sclerites of the Red Sea octocoral *Dendronephthya hemprichi* have been chosen as a model for this study. Water droplet condensation on the sclerites has been *in-situ* investigated using Quanta 200 FEG (field emission gun) ESEM (environmental scanning electron microscope) under wet environmental conditions. Two different analysis methods of droplet top and side views have been applied to determine the contact angle based on the secondary electron images. The ESEM image analysis for the sclerites indicates that their surface is hydrophilic. The microscopic contact angle is measured to be 45.3° ± 6.3°. The macroscopic contact angle has been calculated by using the Wenzel model for the surface texturing of the sclerites.

Key words: Droplet, contact angle, octocorals, *Dendronephthya hemprichi*, ESEM.

1. Introduction

Corals are divided into two: hard corals (stony corals, or scleractinians), which are the major reef-builders featuring rigid calcium carbonate skeleton and octocorals (fleshy soft corals and sea fans), which have numerous tiny sclerites embedded in soft fleshy matrix. The importance of the reefs is as wave breakers, protection against floods, land-erosion and devastating storms such as hurricanes and typhoons. While alive, the reefs provide habitat for a high diversity of organisms of both invertebrates and fish. Coral reefs are also known for their economic importance, being source for food and touristic activities.

Global environmental changes due to fossil fuel burning and other sorts of pollution result with ocean acidification. This phenomenon is caused by emission of CO_2, which in turn forms carbonic acid when dissolved in seawater and thus lowers the ocean pH levels. It thus threatens further calcification of reef-building corals and other calcifiers. Previous work [1] indicated that fleshy tissues of octocorals may act as a barrier against skeleton corrosion under acidic conditions. This was tested against isolated sclerites, which underwent dissolution damage related to water acidity. The erosion damage of octocorals has a tight connection to their wettability properties [2].

The exoskeleton of stony corals which feature high content calcium carbonate scaffolds was shown to be biocompatible, osteo-conductive and biodegradable, which also depends on the porosity, implantation site and the specific coral species. In particular, corals were studied as potential bone graft substitutes in animals in the early 1970s and afterwards in humans in 1979 [3]. The biocompatibility of the calcium carbonate coral skeleton in respect to cell adhesion requires characterization of coral structures on the relevant cell size and in comparable to their surface inhomogeneity scale, i.e., at the micron-scale [4].

In this work, the spindle-shaped sclerites of the Red Sea octocoral *Dendronephthya hemprichi* [2] were studied by measuring the wettability properties on micron-scale. These calcite-made sclerites, which feature wary surface-microstructures, have been

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chosen for this study [5]. The droplet condensation on the sclerites has been *in-situ* imaged in the ESEM (environmental scanning electron microscope) under wet environmental conditions in similarity to the research of Aronov, et at. [6]. At this characteristic scale, the typical drop size is below the capillary length. The effect of gravity is thus neglected and the droplet acquires a perfectly spherical cap shape.

Two different analysis methods for top and side views [7, 8] were applied to determine the microscopic contact angle based on the secondary electron ESEM images. The methods were compared to provide a methodology for micron-scale contact angle derivation on *Dendronephthya* sclerites as depicted at that annual meeting [9]. Macroscopic contact angle measurements were previously reported on flat calcium carbonate surfaces by using calcium and carbonate ions in supersaturated solutions for preparation of calcium carbonate coatings [10]. In the current study, the macroscopic contact angle was calculated by the Wenzel model for rough surfaces [11], using the measured microscopic angle and the surface structure of the sclerites. The calculated macroscopic contact angle value was compared with optical goniometer measurements.

2. Experiments

2.1 Experimental Set Up

Samples of *Dendronephthya hemprichi* were collected from the coral reef across from the Interuniversity Institute for Marine Sciences in Eilat (IUI), Gulf of Aqaba, northern Red Sea. The sclerites were obtained by dissolving the tissues in 10% sodium hypochlorite, followed by careful rinsing in distilled water. The isolated sclerites were dried out and mounted on the ESEM cooling stage.

The current research was focused on *in-situ* micron-scale imaging of calcite-made sclerites. Imaging was performed in the Quanta 200 FEG ESEM using the GSED (gaseous secondary electron detector) with Peltier cooling stage [9, 12]. Sample imaging was done at 2° and 5.3 torr while *in-situ* condensation was performed at an elevated pressure up to 5.8 torr.

2.2 Micro-contact Angle Analysis Methods

Two ESEM image analysis methods have been used to measure the contact angle. The first analysis method corresponds to top view measurement (view parallel to primary electron beam axis). The secondary electron intensity profile along the droplet diameter is based on the model of Stelmashenko, et al. [7] and fitted to Eq. (1):

$$I(\mathrm{x}) = I_0\left(1-\frac{(X-X_\mathrm{C})^2}{a^2}sin^2 w\right)^{-1/2} + I_\mathrm{B} \qquad (1)$$

where, the *w* is contact angle, *a* is the apparent radius of the droplet base, x_c is the position of its center, *I(x)* is the pixel intensity measured from an electron micrograph, I_0 and I_B are the intensity coefficients related to the normal intensity of secondary electron emission and background levels, respectively. The droplet base radii a and the position of droplet center x_c can be read off the image scan for each droplet, when the intensity at the droplet center was taken as $I_0 + I_\mathrm{B}$. The intensity value at the ESEM image across the center of each droplet allows extracting the value of the contact angle *w*, which is the only fitting parameter in Eq. (1). Similar approach was performed for *in-situ* droplet condensation on flat silicon samples before and after surface e-beam treatment for hydrophilic and hydrophobic-like contact angle derivation [6].

The surface of *Dendronephthya hemprichi* sclerites is not flat and can rarely be tilted to produce an exact top view or side view images. Droplets formed on the warty surface are at an arbitrary angle relative to the incident beam. An alternative mathematical model thus provides extracting the real contact angle from SE images for spherical cap drops observed from an arbitrary angle. This model correlates the real contact angle *w*, the measured angle δ, and the substrate inclination angles z versus horizontal direction [8]:

$$w = 2\tan^{-1}\left\lfloor\frac{\sqrt{(tan\,(\delta/2))^2+(\cos z)^2-1}+\tan(\delta/2)}{\cos z+1}\right\rfloor \qquad (2)$$

3. Results and Discussions

3.1 ESEM-Characteristic Morphology

A portion of a typical spindle-shaped sclerite of *Dendronephthya hemprichi* is presented in Fig. 1. It can be seen that the protruding features of the sclerite are roughly 25 μm apart, 10 μm in height and 10 μm in width as schematically presented at Fig. 2.

3.2 ESEM-Wettability Top View Measurement

Upon *in-situ* condensation, droplets appear on the sclerites (Fig. 3a). Some drops were tilted with respect to the top view, while others were usually asymmetrical due to an interaction with a protruding feature or apparent defects on the surface. In comparison, the droplets formed on the carbon flat substrate itself are highly symmetrical and can be analyzed according to Stelmashenko's top view model [7] for contact angle (Fig. 3a). Fig. 4 shows a typical comparison of intensity profile for the droplet on the sclerite surface relative to a reference drop on the carbon substrate (corresponding to two lines at Fig. 3b). The characteristic comparison is a function of position with fit to Eq. (1) for the contact angle derivation. The reference drop on carbon flat surface (Fig. 4a) provided a typical contact angle of 57° carbon, while the specific drop on the sclerite was characterized by asymmetric

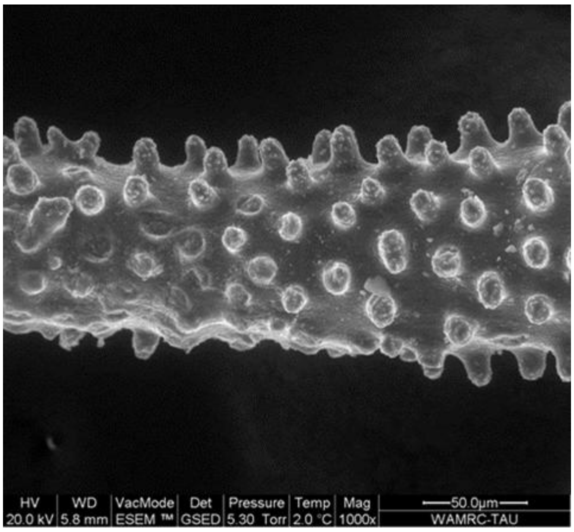

Fig. 1 Spindle shaped sclerite of *Dendronephthya hemprichi* prior droplet condensation.

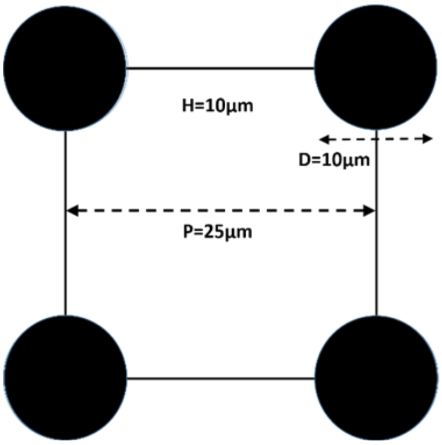

Fig. 2 Schematic basic unit structure of sclerite of the octocoral *Dendronephthya hemprichi*.

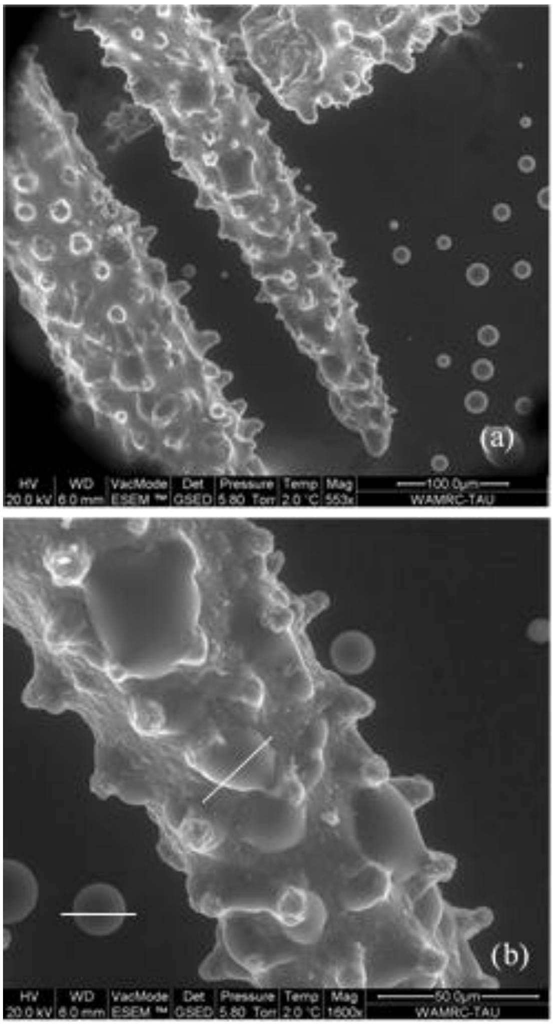

Fig. 3 *In-situ* condensation on sclerite of *Dendronephthya hemprichi*: (a) drops on the sclerite are misshaped in contrast to droplet on the carbon (on the right); (b) a magnified image of the middle part of the sclerite.

contact angle values

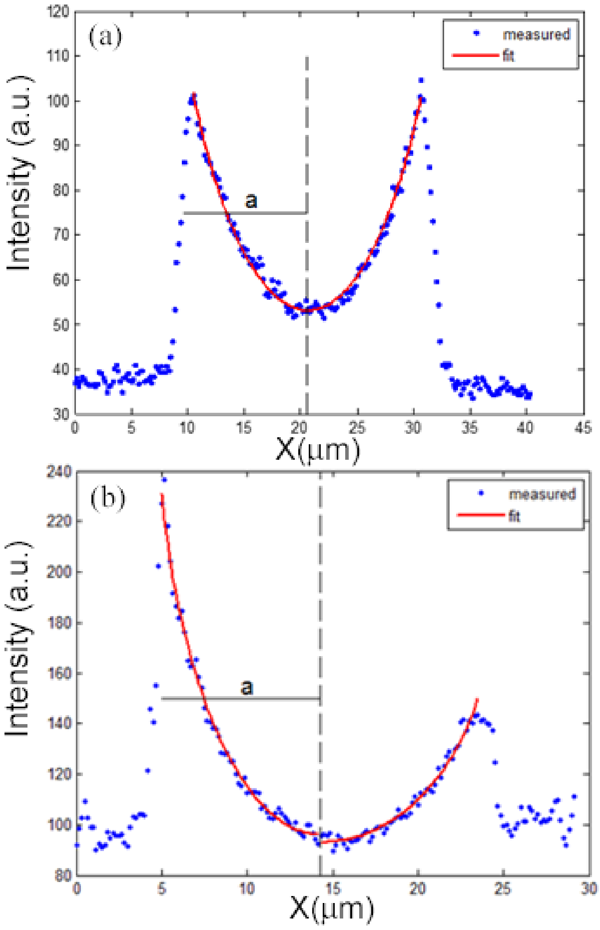

Fig. 4 Gray level intensity (arbitrary units) along the two lines chosen in Fig. 3b: (a) for drop on carbon; (b) for drop on the sclerite of *Dendronephthya hemprichi*.

(of 74° and 65°) for both left and right sides of the profile (Fig. 4b). Additional measurements on the sclerites resulted in wide spread values, which required an alternative evaluation method.

3.3 ESEM-Wettability Side View Measurement

Side view contact angle measurements on octocoral *Dendronephthya hemprichi* sclerites structures are shown in Fig. 5. The real contact angle is extracted by using Brugnara's method for the measured and the substrate inclination angles according to Eq. (2). The results of overall 22 droplets provided a Gaussian distribution with contact angle of 45° ± 6°. The chosen micro-droplets were from regions in-between the protruding features, thus presenting the Young contact angle for a smooth surface structure. This micron-scale measurement thus corresponds to the intrinsic material properties. Using these results, predictions for macroscopic wetting properties, which depend on morphology, could be attained.

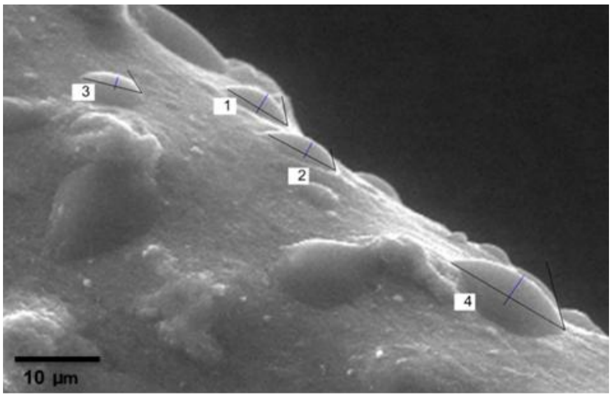

Fig. 5 Side view of four droplets on sclerite of *Dendronephthya hemprichi* showing the measured contact angles.

3.4 Calculation of Macroscopic Contact angle Based on Wenzel Model

The structure was modeled based on the ESEM images and the corresponding schematic basic unit of *Dendronephthya hemprichi* sclerites (Fig. 2). For simplicity, the structure was modeled assuming a two dimensional periodic structure of protruding feature height H = 10 μm, diameter D = 10 μm and separation P = 25 μm. The Young contact angle of w~45° was used for a smooth surface. The apparent macroscopic contact angle w_{m} for the modeled structure was related to the Young angle w by including the roughness effects [13]:

$$\cos w_{\mathrm{m}} = \left(1+\frac{\pi DH}{P^2}\right)\cos w \qquad (3)$$

Substituting: w ~ 45°, D = 10 μm, H = 10 μm, P = 25 μm, provided complete wetting w_m ~ 0 under current assumptions and in correlation to optical goniometer measurements. It is thus indicated that the surface roughness of the octocoral *Dendronephthya hemprichi* improves the wetting properties towards complete wetting.

4. Conclusions

The ESEM provides a high spatial resolution and a relatively large depth of field of tens microns, which has been required for characterization of the rough surface of *Dendronephthya hemprichi* sclerites before and after *in-situ* droplet condensation.

The methodological research for micro-scale droplet measurement on the sclerites was based on comparing the top view and the side view ESEM measurements. The top view method resulted in a wide spread of contact angles due to drop coverage of the inhomogeneous surface. The droplets chosen for analysis in the side view method could far outnumber the droplets which can be analyzed in the top view method. Thus, unlike flat substrates, the *in-situ* side view droplet contact measurement in ESEM was found recommended over top view measurement for the rough surface of the *Dendronephthya hemprichi* sclerites. The statistics of the microscopic contact angle was of Gaussian distribution with mean value of 45.3° and a standard deviation error of 6.3°. This value was within the range of prior work on macroscopic contact angle of calcium carbonate flat surfaces [10].

The chosen micro-droplets at side view measurements were in-between the protruding features of the sclerites, providing the intrinsic wetting properties of the calcite-made sclerites. The micron-scale contact angle of about 45° differs from the macroscopic zero contact angle by optical goniometer. The difference is explained by including the surface roughness using the Wenzel model for apparent contact angle. The results thus show that the sclerite surface roughness of octocoral *Dendronephthya hemprichi* induces complete wetting.

Under non-active sea conditions, the skeleton of the sclerites is completely embedded in the soft fleshy matrix as could be explained by complete wetting, especially for the highly viscous fleshy matrix. Further analysis would include the dependence of the sclerite wetting properties on the sea water pH levels and on the skeleton surface treatments. The microstructures of octocoral sclerites is highly variable [4], which could affect their surface roughness, wettability properties and potential usage as biocompatible materials.

Acknowledgments

The authors thank the Wolfson Applied Materials Center and the center for Nano Science and Nanotechnology for feasibility study in the environmental scanning electron microscope. The study was in part supported by The Israel Cohen Chair in Environmental Zoology to Y.B. The authors thank the Interuniversity Institute for Marine Sciences in Eilat (IUI) for facilities. Collection of animals complied with a permit issued by the Israel Nature and Parks Authority.

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