Catalytic effect of microbially-derived carboxylic acids on the precipitation of Mg-calcite and disordered dolomite: Implications for sedimentary dolomite formation

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ABSTRACT

The genesis of dolomite is a contentious issue partly due to the difficulty in its synthesis at ambient temperature. Certain types of microorganisms have been demonstrated to be effective in promoting the precipitation of disordered dolomite, an important precursor of sedimentary ordered dolomite. In contrast to a growing body of research on the catalytic role of microbial exopolymers in the crystallization of disordered dolomite, the role of other microbial exudates (e.g., carboxylic acids) remains unknown. To fill in this knowledge gap, precipitation experiments, mimicking the carbonation process within microbial mats, were conducted in saline solutions containing 0-30 mM succinic acid or citric acid, which are commonly produced by microbes. The starting salinities of experiment solutions were set to 35‰ and 70‰, in order to evaluate the effect of solution salinity on dolomite formation. Our results showed that both succinic acid and citric acid enhanced the incorporation of Mg$^{2+}$ into growing Ca-Mg carbonates. Solution salinity also played a positive role in enhancing Mg signature in Ca-Mg carbonates. Disordered dolomite with 40.92 mol% MgCO$_3$ was detected under the conditions of 30 mM succinic acid and 70‰ salinity, whereas in other reactors with succinic acid, Mg-calcites formed. Citric acid was more effective in loading Mg$^{2+}$ into Ca-Mg carbonates compared with succinic acid, as evidenced by the predominant occurrence of disordered dolomite with MgCO$_3$ content ranging from 40.91 mol% to 46.75 mol% in most conditions tested. The results of this study have implications for the formation mechanism of sedimentary dolomite.

Keywords: dolomite problem; disordered dolomite; carboxylic acid; microbial
1. Introduction

The mineral dolomite was firstly discovered by Déodat de Dolomieu in 1791 and was later characterized by having a chemical composition of \( \text{CaMg(CO}_3\text{)}_2 \) and by consisting of alternating layers of pure Mg and Ca ions separated by layers of \( \text{CO}_3 \) (Gregg et al., 2015). However, it has been recognized that sedimentary dolomite exhibits variations in chemical composition and Ca-Mg order (Land, 1985; Warren, 2000). In general, the composition of natural dolomites ranges from \( \text{Ca}_{1.16}\text{Mg}_{0.84}(\text{CO}_3)_2 \) to \( \text{Ca}_{0.96}\text{Mg}_{1.04}(\text{CO}_3)_2 \) (Warren, 2000). Moreover, dolomite crystals in nature possess partially or fully ordered structure (Goldsmith and Graf, 1958; Land, 1985; Warren, 2000; Gregg et al., 2015).

Despite the fact that the chemical and textural properties of dolomite have been well studied, the mechanism of its formation remains controversial (Warren, 2000; McKenzie and Vasconcelos, 2009). Such debate, often known as “dolomite problem”, was driven by the sharp contrast of the common occurrence of dolomite in pre-Holocene geological records to its paucity in modern sediments and by the difficulty in its synthesis in ambient laboratory settings (Land, 1998; Arvidson and Mackenzie, 1999; Warren, 2000). However, precipitation of dolomite in saturated solution (e.g., modern seawater) is thermodynamically favorable (Given and Wilkinson, 1987). A satisfactory explanation for the scarce occurrence of modern dolomites offered by laboratory experiments further shows that dolomite crystallization is a kinetically-controlled
process (Baker and Kastner, 1981; Lippmann, 1982; Shen et al., 2014). Among reported controlling factors, the hydration effect of Mg$^{2+}$ has been recognized as a key kinetic barrier to the incorporation of Mg$^{2+}$ into the lattice of dolomite (Zhang et al., 2012a, b; Shen et al., 2014). Hydrothermal experiments also indicate that the kinetic barrier to dolomite can be overcome at higher temperatures (>100 °C) and disordered dolomite appears as a metastable precursor to dolomite (Malone et al., 1996; Zhang et al., 2010; Rodriguez-Blanco et al., 2015; Kaczmarek and Thornton, 2017). In comparison to dolomite (space group: R3), disordered dolomite (whose space group is R3c) has near-dolomite stoichiometry (more than 36 mol% and up to ca. 55 mol% MgCO$_3$) but completely disordered Ca-Mg arrangement (Fang and Xu, 2019). On the other hand, disordered dolomite has the same space group as calcite, but its Mg content is higher than that of Mg-calcite (<36 mol% MgCO$_3$) (Fang and Xu, 2019).

Although modern dolomite is rarely found in open marine sediments, primary (or very early diageneric) dolomite precipitates that apparently formed at low temperatures (<60 °C) have been repeatedly observed in highly evaporitic environments (e.g., Vasconcelos and McKenzie, 1997; Wright, 1999; van Lith et al., 2002, 2003a; Wright and Wacey, 2005; Bontognali et al., 2010, 2012; Deng et al., 2010; Meister et al., 2011; Fang and Xu, 2019; Liu et al., 2019a) and marine cold seeps (e.g., Xu et al., 2010; Lu et al., 2018). These dolomites are normally non-stoichiometric and poorly ordered (Petrash et al., 2017; Fang and Xu, 2019). Laboratory experiments have further shown that the activities of certain types of microorganisms (e.g., sulfate-reducing bacteria, methanogens and halophilic aerobic microbes) are intimately associated with these
precipitates (Vasconcelos et al., 1995; Warthmann et al., 2000; van Lith et al., 2003b; Roberts et al., 2004; Sánchez-Román et al., 2008, 2009, 2011; Kenward et al., 2009; Xu et al., 2010; Qiu et al., 2017; Liu et al., 2019a). Recently, a growing body of research has revealed the positive role of microbial exopolymers (EPS) in dolomite crystallization (Bontognali et al., 2008, 2014; Krause et al., 2012; Zhang et al., 2015; Liu et al., 2020). Microbial EPS and other biomolecules with high carboxyl-group densities have been demonstrated to effectively diminish the aforementioned hydration effect of Mg$^{2+}$ and thus expedite dolomite precipitation (Kenward et al., 2013; Roberts et al., 2013; Zhang et al., 2015).

It is important to note, however, that Gregg et al., (2015) reevaluated the published XRD data of the reportedly microbially-induced dolomites and found that these carbonates actually lack cation ordering. Therefore, disordered dolomite rather than presumably reported dolomite is synthesized by means of microbial mediation (Gregg et al., 2015). Nevertheless, as pointed before, disordered dolomite is considered as the precursor for some ordered dolomites in sedimentary rocks (Warren, 2000; Zhang et al., 2012a, b; Rodriguez-Blanco et al., 2015). In this regards, the contribution of microbes and their organic secretions (e.g., EPS) to the genesis of dolomite in subsurface sediments and sedimentary rocks cannot be ruled out.

In addition to the long-chain EPS, microorganisms also secrete copious low-molecular-weight (LMW) organic acids into solution (Sauer et al., 2008). There are several studies that note the positive role of LMW organic acids (e.g., acetate) in the uptake of Mg into growing Mg-calcites (Xu et al., 2010; Zhang et al., 2010). However,
it is still unclear whether microbially-derived carboxylic acids can aid in the formation
of disordered dolomite. In this study, we examined the synthetic formation of Ca-Mg
carbonates using supersaturated solutions containing succinic acid ($C_4H_6O_4$) or citric
acid ($C_6H_8O_7$). The reason for these two carboxylic acids tested in this study was that
they can be produced by various microorganisms in the tricarboxylic acid cycle (TCA
cycle) (Sauer et al., 2008) and thus be virtually found in most aqueous settings,
especially within microbial mats where microbial activities are prevalent. Additionally,
the effect of salinity on carboxylic acid-mediated precipitation of Ca-Mg carbonates
was also evaluated in this study because the salinity in ocean dynamically changed
throughout geological history (Knauth, 2005; Hay et al., 2006) and is considered as one
of key controls over dolomite crystallization (Folk and Land, 1975). Our results showed
that both LMW carboxylic acid and solution salinity could promote the uptake of $Mg^{2+}$
into precipitating Ca-Mg carbonates, thus forming Mg-calcite and even disordered
dolomite at 25 °C.

2. Materials and methods

2.1. Synthesis of Ca-Mg carbonates from carboxylic acid-containing solutions and
chemical analyses

An apparatus using the ammonium carbonate free-drift technique described by
Lian et al., (2006) was employed in our experiments. Unlike most conventional
methods for synthesizing Ca-Mg carbonates, in which the cations of calcium and
magnesium and carbonate anions are mixed instantaneously and simultaneously, the
minerals in our experiments formed by gradually delivering carbonate into the Ca/Mg solution. The purpose of such operation is mimicking the carbonation process occurring within microbial mats, in which microbial degradation of organic matters (e.g., proteinaceous substances) could continuously provide NH$_4^+$ and CO$_3^{2-}$ to benefit the precipitation of Ca-Mg carbonates.

Briefly, a number of 100-mL conical glass flasks containing experimental solutions were placed into the upper space of a closed desiccator, along with three underlying beakers each holding 15 g of solid ammonium bicarbonate (NH$_4$HCO$_3$). The experimental solutions consisted of 15 mM CaCl$_2$, 90 mM MgCl$_2$·6H$_2$O, different concentrations of succinic or citric acid (0, 5, 10, 20 and 30 mM; Sigma-Aldrich Chemical Company, $\geq$ 99.5%) and various amounts of NaCl to obtain starting salinity of 35‰ or 70 ‰. It is relevant to note that the starting Mg/Ca molar ratio is higher than that of modern seawater (6 vs. 5.2), but lies within the range of values measured in present dolomite-forming environments (Table 1 in Deng et al., 2010). The pH of the solutions was adjusted to $\sim$7.50 by addition of 0.5 M NaOH solution. The desiccators were placed in an incubator at 25 °C. Once experiments have been set up, steady-state decomposition of NH$_4$HCO$_3$ could provide NH$_3$ and CO$_2$ gas, which diffused into the aforementioned solutions and then simultaneously increased solution pH and carbonate alkalinity. As such, growth of Ca-Mg carbonate could occur in the flasks. All experiments were run in duplicates. After two weeks, the precipitates were carefully collected by centrifugation, rinsed three times with doubly distilled water (ddH$_2$O), and then freeze-dried.
Solution pH and aqueous Ca\(^{2+}\)/Mg\(^{2+}\) were measured during carbonation. The pH was determined using a Denver UB-7 pH meter (Denver, USA). The concentrations of Ca\(^{2+}\) and Mg\(^{2+}\) were analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES, Thermofisher ICAP6300, USA).

2.2. Preparation of carbonate standards

As hydromagnesite \([\text{Mg}_5(\text{CO}_3)_4(\text{OH})_2\cdot4\text{H}_2\text{O}]\) can be easily misidentified as disordered dolomite or dolomite (Zhang et al., 2012a), a hydromagnesite sample, together with inorganic calcite, disordered dolomite and dolomite, was prepared as a standard for inferring the mineralogical composition of our solid products (Liu et al., 2019b). Hydromagnesite was purchased from Xibeili mineral company (Jiangsu, China) (Liu et al., 2019b). The precipitation of calcite was carried out in a glass vessel at 25 °C according to published methodologies (e.g., Butler et al., 2006). Disordered dolomite and dolomite were prepared at 80 °C and 200 °C, respectively, following a reported protocol (Rodriguez-Blanco et al., 2015).

2.3. Mineral analyses

Multiple methods were used for mineral analyses. The mineralogical compositions of the precipitates were identified by X-ray diffraction (XRD) and JADE 6 program (MDI, Livermore, USA). Scanning electron microscopy (SEM) and attached energy-dispersive X-ray spectroscopy (EDS) were employed for morphology observations and chemical composition analyses. The crystal structure of precipitates was examined by transmission electron microscopy (TEM) and Raman spectroscopy.

Specifically, all XRD analyses were performed using a Scintag X1 X-ray powder
diffractometer (Scintag, Inc., USA) equipped with Cu-Kα radiation at 40 kV and 35 mA. The samples were scanned from 5 to 65° 2θ with a scan rate of 2° 20/min. The MgCO₃ contents of Ca-Mg carbonates were calculated from the d-spacing of the (104) peak by using the calibration curve of Bischoff et al., (1983). The resulting precipitates were Pt-coated prior to be characterized by a Hitachi SU8010 SEM (Hitachi, Inc., Japan). The SEM was operated at an accelerating voltage of 5-15 kV. For the TEM observations, samples were suspended in ethanol and then pipetted onto 300-mesh formvar-coated Cu grids. Transmission electron micrographs of the samples were recorded by a JEOL JEM-2100 LaB6 TEM (JEOL, Japan) with a 200 kV accelerating voltage. Quantitative EDS and selected area electron diffraction (SAED) were employed to characterize the individual crystal structure and atomic composition. TEM images were collected and processed by Gatan DigitalMicrograph software. Raman spectra were taken by using a RM-1000 laser Raman microscope system (Renishaw, UK) in the range between 100 and 2000 cm⁻¹. Excitation was with a 20 mW 514.5 nm Ar⁺ laser. The scanning parameter for each spectrum was taken as 10 scans, 10 s per wavelength on the detector and a 15 μm slit. Spectral peak positions were calibrated using the 520.5 cm⁻¹ line of silicon as a standard.

3. Results

3.1. Changes of aqueous chemistry as a result of carbonation

The NH₄HCO₃ powders were completely decomposed after two weeks. The reactors with the salinity of 35‰ were selected as representatives to show the time
course changes in aqueous chemistry (Figs. 1 and 2). The pH changes of carboxylic acid-amended systems exhibited a similar trend to those of control reactors (carboxylic acid-free groups): they rapidly increased within the first 10 days and slightly declined afterwards (Figs. 1A and 2A). Similar patterns of calcium removal from solutions were observed in the reactors with or without carboxylic acids (Figs. 1B and 2B), that is, the concentrations of soluble Ca$^{2+}$ ions gradually decrease from ca. 15 mM to near zero by day 14. Unlike Ca$^{2+}$ ions, the changes in concentration of Mg$^{2+}$ were quite different between control and carboxylic acid-amended systems (Figs. 1C and 2C). In general, there was no appreciable Mg$^{2+}$ depletion in control sets. By contrast, the declines in Mg$^{2+}$ ions were observed when carboxylic acids were introduced into the precipitation reactors and these decreases were apparently related to increased dosage of carboxylic acid in solution. Interestingly, citric acid was more effective in removing Mg$^{2+}$ from solutions than succinic acid at the same concentration. Furthermore, in carboxylic acid-amended groups, the concentrations of precipitated Mg (depletion of Mg$^{2+}$) were linearly correlated with the concentrations of precipitated Ca (Figs. 1D and 2D).

3.2. Characterization of the produced Ca-Mg carbonates

3.2.1. XRD results

During the carbonation process, cloudy solutions were observed within 12 h in the control systems and in 24-48 h in the presence of succinic or citric acids, respectively. Upon crystallization over two weeks, the solid products were collected and then investigated using XRD to probe the effect of LMW carboxylic acids on the crystallization of carbonates. The results showed that the crystals formed in the control
systems with salinities of 35‰ and 70 ‰ were exclusively aragonite (CaCO$_3$) (Fig. 3).

As expected, the products obtained changed obviously when succinic or citric acid was introduced into the experimental solutions (Figs. 4 and 5). Specifically, the Ca-Mg carbonates of calcite-dolomite series were identified as the major phases, exhibiting the characteristic reflection of a rhombohedral crystal [(hkl) Miller indices: (012), (104), (006), (110), (113), (202), (018), (116), etc.]. In addition to these Ca-Mg carbonates, aragonite and monohydrocalcite (CaCO$_3$·H$_2$O) were also produced in some cases.

Compared the XRD patterns of Ca-Mg carbonates obtained in each set, shifts in d-spacing could be clearly observed, indicating that different amounts of Mg$^{2+}$ were incorporated into their crystal lattices. To better illustrate the relationship between the concentrations of carboxylic acids and MgCO$_3$ contents in Ca-Mg carbonates, short XRD scans of the (104) reflections for newly formed phases were further obtained (Figs. 4 and 5). These XRD profiles revealed that the (104) peak of Ca-Mg carbonate became broader and showed a much higher peak intensity with increasing concentrations of succinic (Figs. 4B and 4D) or citric acid (Figs. 5B and 5D). More interestingly, it also showed that increment in the concentration of these compounds could lead to a progressive decrease in the (104) d-spacing of Ca-Mg carbonate. As clearly shown in Figure 6, the (104) d-spacing (corresponding to mol% MgCO$_3$ in the crystallites) was significantly correlated with the concentration of succinic or citric acid, indicating that these biomolecules are effective in promoting Mg uptake into calcitic structures. It is also interesting to note that the influence of carboxylic acids not only acts in a concentration-dependent fashion, but is also highly dependent on their type. For
example, when the solution salinity was 35‰, the carbonates with 8.17, 10.35, 28.91 and 33.28 mol% MgCO$_3$ precipitated from solutions with 5, 10, 20 and 30 mM succinic acid, respectively (Bischoff et al., 1983). However, the catalytic effect of citric acid over the same concentration range became more obvious: produced carbonates with an average MgCO$_3$ content of 9.62, 40.92, 42.01 and 44.56 mol%, respectively (Figs. 6B and 6D). Such phenomenon was also found in the experimental systems with the salinity of 70‰. Furthermore, it seems that the elevated salinity of carboxylic acid-bearing solution could also enhance the substitution of Mg for Ca within the resulting carbonate phases, especially those that precipitated from the succinic acid-amended reactors (Fig. 6).

According to the terminology proposed by Fang and Xu (2019), the substitution of Mg for Ca sites in calcitic structures can result in four calcite derivatives, including low-Mg calcite (< 4 mol% MgCO$_3$), high-Mg calcite (ranging in content from 4 mol% to 36 mol% MgCO$_3$), disordered dolomite with near-dolomite stoichiometry (more than 36 mol% and up to ca. 55 mol% MgCO$_3$) but lacking cation ordering, and dolomite. Employing such criteria, the Ca-Mg carbonates formed in the 5-30 mM succinic acid-amended systems with the salinity of 35‰ had MgCO$_3$ composition ranging from 8.17 mol% to 33.28 mol%, corresponding to high-Mg calcite (Fig. 6). Once the salinity of succinic acid-bearing solutions increased up to 70‰, the highest MgCO$_3$ content of Ca-Mg carbonates produced in the solution with 30 mM succinic acid can reach 40.92 mol%, close to that of dolomite, while Mg-calcites with MgCO$_3$ content ranging from 11.08 mol% to 31.82 mol% precipitated in other systems. As stated before, the MgCO$_3$
contents of synthetic Ca-Mg carbonates facilitated by citric acid were significantly
higher than those by succinic acid. Ca-Mg carbonates close to dolomite composition
(covering the range 40.91-46.75 mol% MgCO₃) were produced in the solutions with
citric acid over the concentration range of 10-30 mM either at the salinity of 35‰ or at
70‰, and Mg-calcites formed when only 5 mM citric acid was used.

To exclude the possible misidentification and determine the degree of cation
ordering in our organogenic dolomite-like minerals, the XRD reflections of Ca-Mg
carbonates induced by 30 mM carboxylic acids, along with inorganically-synthesized
disordered dolomite, ordered dolomite, calcite and hydromagnesite, were further shown
in Figure 7 for comparison. The XRD pattern and peak position of dolomite-like phases
catalyzed by succinic or citric acid were quite similar to those of inorganic disordered
dolomite precipitated at 80 °C. In addition, there were no ordering reflections [e.g.,
(015), (021), and (101)] in XRD patterns of carboxylic acid-mediated dolomite-like
phases. As such, above features indicate that the crystal structure of these dolomite-like
materials was highly disordered and thus that they can be identified as disordered
dolomites.

In summary, these data indicated that (i) both carboxylic acid and salinity had a
considerable impact on the Mg²⁺ uptake into calcitic structures, leading to the
precipitation of Mg-calcite and even disordered dolomite at ambient temperature; (ii)
in comparison to succinic acid, citric acid was more effective in enhancing Mg
incorporation into Ca-Mg carbonates.

3.2.2. Micro-Raman analyses
Raman spectra in the range of 130-1200 cm\(^{-1}\) were used for interpreting the structural differences among organogenic disordered dolomite, inorganic disordered dolomite and dolomite, and calcite. The spectral bands of rhombohedral crystals in this low-wavenumber region have been widely studied and successful assignments of these vibrations (e.g., Bischoff et al., 1985; Edwards et al., 2005; Perrin et al., 2016).

As shown in Figure 8A, similar patterns with four distinct bands were observed in our samples, as consistent with previous studies of carbonate minerals (Bischoff et al., 1985; Perrin et al., 2016). These Raman bands consisted of two lattice modes (i.e., T and L external vibrations) below 350 cm\(^{-1}\) that are attributed to motions comprising the complete unit cell (Wehrmeister et al., 2010) and two other internal modes (i.e., \(\nu_1\) and \(\nu_4\)), which are due to the symmetric stretching or in-plane bending of the C-O bonds in the carbonate ion (Wehrmeister et al., 2010; Perrin et al., 2016). Specifically for each inorganic standard (Fig. 8A), the peak in the T region was at 155 cm\(^{-1}\) for calcite, 173 cm\(^{-1}\) for disordered dolomite and 175 cm\(^{-1}\) for dolomite; the position of the L peak appeared at 281 cm\(^{-1}\) for calcite, 297 cm\(^{-1}\) for disordered dolomite and 300 cm\(^{-1}\) for dolomite; the peak in the \(\nu_4\) region observed for these three phases was at 711, 723 and 723 cm\(^{-1}\), respectively; in the \(\nu_1\) cases, the peak position for above standards appeared at 1086, 1095 and 1097 cm\(^{-1}\), respectively. These observations are in good agreement with previous studies (Bischoff et al., 1985; Perrin et al., 2016). In comparison to the standards, the band positions of our carboxylic acid-induced precipitates were much close to that of inorganic disordered dolomite (Fig. 8B), again suggesting that the cation occupancy in the carboxylic acid-catalyzed Ca-Mg carbonate was disordered rather...
than ordered.

3.2.3. SEM observations

The solids produced in our precipitation experiments with 30 mM carboxylic acid at the salinity of 35‰ or 70‰ were selected as representatives for SEM investigation. Mixture-shaped particles with various sizes were obtained under a condition of 30 mM succinic acid and 35‰ salinity (Fig. 9A). These solid phases mainly included elongated rods and spheroidal aggregates (Fig. 9B). The corresponding EDS results (Figs. 9C and 9D) revealed that the rod-like microcrystal (particle a in Fig. 9B) contained abundant Ca but negligible Mg, whereas the spheroidal phase (particle b in Fig. 9B) displayed a relatively high Mg/Ca ratio. Combined with XRD data, these two types of solid phases were identified as aragonite and high-Mg calcite. At higher magnification, the submicron-sized spheres were found to be uniformly distributed on the surface of aragonite (Fig. 9E). When the solution salinity was as high as 70‰, more aggregated spheroidal particles were produced in succinic acid-containing solution (Fig. 9F).

Detailed observations showed that the spheres had a granular texture itself comprised of many spherical nano-crystals (Fig. 9G). The EDS spectrum (Fig. 9G) showed that these micro-spheroidal neoformations consisted of similar Mg and Ca contents (Kα line area), thus were interpreted as disordered dolomites. SEM images revealed that the solid products in the reactors with 30 mM citric acid were spherulites with averaged size of 2-4 μm (Fig. 10). Based on EDS analyses and XRD results, these spherulites occurring at salinities of 35‰ or 70‰ were identified as disordered dolomites (Fig. 10).

Higher magnification images showed that disordered dolomite spherulites were
composed of numerous nanoparticles (the inserts in Figs. 10B and 10D), similar to those obtained in succinic acid-bearing systems.

### 3.2.4. TEM observations

TEM was employed to capture the structure of carbonate minerals occurring in the reactors under a condition of 30 mM carboxylic acid and 35‰ salinity (Figs. 11 and 12). The occurrence of rod-like aragonite in succinic acid-containing groups was collectively verified by the results of TEM image, SAED pattern and EDS elemental composition (Figs. 11A-C). In addition to aragonite, high-Mg calcite was also detected (Figs. 11D-G), in good agreement with XRD and SEM results. As shown in Fig. 11D, high-Mg calcite appeared as spheroid-shaped aggregates. High resolution TEM (HRTEM) image further revealed that these nano-sized spheroids were randomly distributed (Fig. 11G).

The disordered dolomite crystal obtained from citric acid-containing groups was identified by its morphology and chemical composition from an EDS spectrum (Fig. 12A). The disordered dolomite spheroid, when magnified, displayed randomly oriented nano-crystals as shown in Fig. 12B, consistent with the SEM observations (Figs. 10A and 10B). A representative SAED pattern of this sample showed the presence of (104), (110), (113) and (024) reflections but lack of the typical super-lattice reflections [e.g., (015), (101) and (021)] (the insert in Fig. 12B). Lattice fringe image was further obtained from the edge site of one disordered dolomite particle. It can be found that our synthetic disordered dolomite had a dominant spacing of 0.290 nm, corresponding to the d-spacing of (104) (Fig. 12C).
4. Discussion

4.1. The possible role of carboxylic acid in the crystallization of Mg-calcite and disordered dolomite

Many factors control formation of primary Ca-Mg carbonates (e.g., Mg-calcite and disordered dolomite). Once oversaturated condition is achieved, the intrinsic chemical property of Mg$^{2+}$ ion becomes a key factor affecting the growth of Ca-Mg carbonates (Lippman, 1973; de Leeuw and Parker, 2001; Romanek et al., 2009; Shen et al., 2014, 2015). Like Ca$^{2+}$ and other cations, Mg$^{2+}$ ions are strongly hydrated in solution, forming the Mg$^{2+}$-H$_2$O complex (i.e., [Mg(H$_2$O)$_6$]$^{2+}$) (Lippman, 1973). However, due to the larger hydration enthalpy of Mg$^{2+}$ than that of Ca$^{2+}$ (1926 kJ/mole vs. 1579 kJ/mole) (Lippman, 1973), the occurrence of more stable inner-sphere hydration shell around Mg$^{2+}$ ions not only hinders the adsorption of Mg$^{2+}$ onto crystal surface, but also blocks the subsequent binding of carbonate to surface Mg$^{2+}$ (Shen et al., 2014, 2015). In contrast, it is easier to overcome the hydration barrier surrounding Ca$^{2+}$ ions. Such inhibitory effect of Mg$^{2+}$ hydration on the formation of Ca-Mg carbonates has been demonstrated by several computational studies (e.g., de Leeuw and Parker, 2001; Di Tommaso and de Leeuw, 2010). As such, aragonite (a low-Mg calcium carbonate) rather than Ca-Mg carbonates is the dominant carbonate phase when the Mg/Ca molar ratio of saturated solution exceeds 4.0 (Shen et al., 2014, 2015). Such mechanism helps to explain the paucity of dolomite in marine sediment and the predominant occurrence of aragonite in our control sets.
A growing body of studies has suggested that microbial EPS could act as natural
catalysts to promote the incorporation of Mg$^{2+}$ into growing Ca-Mg carbonates (Zhang
et al., 2012a, 2015; Bontognali et al., 2014; Kenward et al., 2013; Liu et al., 2020).
Furthermore, carboxyl moieties within EPS matrix were identified as the major
functional groups to accelerate Mg$^{2+}$ dehydration (Wang et al., 2009; Roberts et al.,
2013; Zhang et al., 2015). Previous studies also proposed that the dehydration reaction
induced by carboxyl moieties proceeds possibly via a metal-chelation mechanism
(Romanek et al., 2009; Roberts et al., 2013). Specifically, the electronegative carboxyl
groups bind to [Mg(H$_2$O)$_6$]$^{2+}$ ion pairs and form a [Mg(H$_2$O)$_5$(R-COO)]$^+$ complex,
along with the ejection of one water molecule (Roberts et al., 2013). Upon the
dewatering, the newly formed Mg-carboxyl complex requires significantly lower
energy when binding CO$_3^{2-}$ than [Mg(H$_2$O)$_6$]$^{2+}$ (Roberts et al., 2013; Shen et al., 2014).
In doing so, it is believed that a thin Ca-Mg carbonate template could form and its
growth will take place when a supersaturated condition is maintained (Roberts et al.,
2013).

Our present study demonstrated that Ca-Mg carbonates occurred in the reactors
with succinic acid or citric acid. Given their multicarboxylic status, these two
biomolecules may function in the same capacity as EPS through aforementioned model
to catalyze the crystallization of Ca-Mg carbonates. This mechanism is likely confirmed
by our observation that citric acid displayed more effective in loading Mg$^{2+}$ into
growing carbonate, resulting in the formation of disordered dolomite in most conditions
tested, because citric acid has higher metal-binding capacity than succinic acid in bulk
solutions (binding constants $K$ of citric acid: $K_{\text{Mg}}=10^{3.37}$, $K_{\text{Ca}}=10^{3.5}$; $K_{\text{succinic acid}}$:

$K_{\text{Mg}}=10^{1.2}$, $K_{\text{Ca}}=10^{1.2}$) (Cannan and Kibrick, 1938; Wang et al., 2009).

In addition to the aforementioned metal-chelation mechanism, the polar behavior of carboxylic acids might also be important for their catalytic role in Ca-Mg carbonate formation. In comparison to water molecules, LMW carboxylic acids typically have a lower dielectric constant ($\varepsilon$). For instance, the $\varepsilon$ value of pure succinic acid is 2.4 at 20 °C, while water molecular has a value of 80.4 under the same temperature (Kirkwood and Westheimer, 1938). It has been well-known that both water activity and desolvation energy of cations can be reduced by adding a component with a low-dielectric constant (Oomori and Kitano, 1987; Zhang et al., 2012a, b). Furthermore, there have been several studies showing the enhancement of Mg levels in growing Mg-calcite or disordered dolomite by some organic additives that exhibit a low Mg-binding affinity, such as methanol, alcohol, propanol, dioxane, methane, acetate and agar (Oomori and Kitano, 1987; Falini et al., 1996; Xu et al., 2010; Zhang et al., 2010; Zhang et al., 2012a). These organic compounds share the same polar behavior, that is, they all have a low-dielectric constant. As such, it is reasonable to speculate that the effective role of LMW carboxylic acids in the formation of Mg-calcite and disordered dolomite, partly ascribed to their polar behavior, can disturb the association between Mg$^{2+}$ and water dipoles.

4.2. The effect of solution salinity on the formation of Ca-Mg carbonates

In addition to the organic molecules with low-dielectric constant or high Mg$^{2+}$-binding capacity, there might be some inorganic factors influencing Mg dehydration.
Among them, the role of temperature has been long recognized, because the inorganically synthesis of disordered dolomite or dolomite exhibits a positive temperature dependence (e.g., Arvidson and Mackenzie, 1999). Apart from temperature, the importance of solution salinity has been repeatedly underscored by the fact that the formation of Mg calcite and (or) non-stoichiometric dolomite is generally found in hypersaline settings, as stated earlier. One explanation for the positive role of salinity in Ca-Mg carbonate formation is that high salinity itself may directly facilitate the dehydration of Mg$^{2+}$ (Lippman, 1973; Machel and Mountjoy, 1986). Once other cations are dissolved into saturated solution, these cations could also interact with water molecules to form metal-H$_2$O clusters (e.g., [Na(H$_2$O)$_n$]$^+$) (Tsurusawa and Iwata, 1999), possibly disturbing the existing Mg$^{2+}$-H$_2$O association. In addition, an indirect effect of salinity has attracted more attention recently. Generally, it is suggested that solution salinity can regulate microbial community composition at first, thus giving rise to the predominance of halophilic microbes, which are thought to be involved in the crystallization of Ca-Mg carbonates, as their cell surface and EPS contain high density of carboxyl groups (Rivadeneyra et al., 2000; Sánchez-Román et al., 2008; Balci and Demirel, 2016; Qiu et al., 2017; Liu et al., 2019a).

Our present results showed that solution salinity had a considerable influence on the formation of Ca-Mg carbonates in the abiotic reactors, perhaps supporting its direct impact on the disturbance of Mg$^{2+}$-H$_2$O association. However, further computational studies are warranted to completely verify such hypothesis.

4.3. Geological implications
Our results revealed that microbially-derived carboxylic acids were effective in diminishing the hydration effect of \( \text{Mg}^{2+} \) and thus promoting the incorporation of \( \text{Mg}^{2+} \) into growing Ca-Mg carbonates. Carboxylic acids are ubiquitous and important components of the aquatic systems, which have been detected in some seawaters and marine porewaters at concentrations of up to ~ 3 mM (Albert and Martens, 1997). Within microbial mats of evaporated saline deposits (e.g., coastal lagoons, brackish and saline lakes), the concentration of carboxylic acids should be higher, because microorganisms usually produce carboxyl-rich compounds to maintain their metabolic functions in the presence of high concentration of salts (Fukuchi et al., 2003; DasSarma, 2006). Even if the concentration of LMW carboxylic acids in microbial mats are not as high as the ones we tested herein, these compounds, together with other microbial exudates (e.g., EPS), might play an important role in the formation of disordered dolomite. Once disordered dolomite has formed, it undergoes an “ageing” process upon burial and converts to well-crystallized ordered dolomite, as documented in previous work (Malone et al., 1996; Warren, 2000; Bontognali et al., 2014; Rodriguez-Blanco et al., 2015). As such, a two-stage model, which begins with the precipitation of disordered dolomite via microbial exudates (EPS and LMW carboxylic acids) followed by subsequent diagenetic transformation of disordered dolomite to ordered dolomite, provides one possible interpretation on the occurrence of dolomite in modern hypersaline environments. Indeed, a field investigation conducted by Bontognali et al. (2010) showed that authigenic dolomites in the sabkha of Abu Dhabi exclusively occurred within the buried microbial mats, in which the concentration of microbial...
exudates was apparently higher than that in non-mat sediments. Such model might also
explain dolomite formation in the ancient sea. It has been postulated that the ocean
salinity in the Precambrian, the “age of microorganisms”, was in the range of 40-70‰,
significantly higher than that of modern ocean (~29‰) (Knauth, 2005). The high
salinity is able to not only initiate the dehydration of Mg-H$_2$O complexes both directly
and indirectly, but also decrease oxygen solubility in seawater (Knauth, 2005) that
benefits the preservation of microbial exudates and dolomite precipitation.

5. Conclusion

We demonstrated that the incorporation of Mg$^{2+}$ into growing Ca-Mg carbonates
in saline solutions could be enhanced by succinic and citric acid and the incorporation
amounts of Mg$^{2+}$ was correlated with the concentration of carboxylic acids and solution
salinity. Disordered dolomites were observed when both 30 mM succinic acid and
salinity level of 70‰ were used, in most systems with citric acid as confirmed by XRD
patterns, Raman spectra and TEM-SAED. Considering the wide distribution of
carboxylic acids and other microbial exudates (e.g., EPS) in natural environments and
high salinity in evaporitic conditions, the microbial exudate-mediated dolomite
formation offers one overlooked possibility for the occurrence of dolomite in Holocene
sediments and sedimentary rocks.

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**Figure caption:**

**Figure 1.** Aqueous chemistry data with time in the experiments with 30 mM succinic acid (SA) at the salinity of 35‰: (A) pH; (B) soluble Ca; (C) soluble Mg; (D) precipitated Ca vs. precipitated Mg.

**Figure 2.** Aqueous chemistry data with time in the experiments with 30 mM citric acid (CA) at the salinity of 35‰: (A) pH; (B) soluble Ca; (C) soluble Mg; (D) precipitated Ca vs. precipitated Mg.

**Figure 3.** XRD patterns of solid products in the control systems at the salinities of 35‰ and 70‰.

**Figure 4.** XRD patterns of minerals obtained from the reactors with 5-30 mM succinic acid: (A) results for the sets at 35‰ salinity and (B) enlarged and smoothed patterns for the (104) reflection; (C) results for the sets at 70‰ salinity and (D) enlarged and smoothed patterns for the (104) reflection (A, aragonite; M, monohydrocalcite).

**Figure 5.** XRD patterns of minerals grown in the presence of different concentrations of citric acid: (A) results for the sets at 35‰ salinity and (B) enlarged and smoothed patterns for the (104) reflection; (C) results for the sets at 70‰ salinity and (D) enlarged and smoothed patterns for the (104) reflection (A, aragonite; M, monohydrocalcite).
**Figure 6.** Plots showing the relationships of carboxylic acid concentration with the (104) d-spacing of Ca-Mg carbonate and with the Mg content in Ca-Mg carbonate: (A-B) succinic acid sets; (C-D) citric acid sets.

**Figure 7.** (A) Comparison of the XRD patterns from organogenic disordered dolomites, inorganically-synthesized disordered and ordered dolomite, calcite and hydromagnesite (SA, succinic acid; CA, citric acid).

**Figure 8.** (A) Raman spectra of organogenic disordered dolomites and inorganic carbonate standards: a-c, disordered dolomites synthesized with aid of 30 mM succinic acid (35‰ salinity), 30 mM citric acid and salinity of 35‰, and 30 mM citric acid and salinity of 70‰, respectively; d, inorganic disordered dolomite; e, calcite; f, ordered dolomite. (B) Comparison of the Raman bands from organogenic disordered dolomites and inorganic carbonate standards.

**Figure 9.** SEM images and SEM-EDS composition show the solid products in the systems with 30 mM succinic acid: (A-E) under the salinity level of 35‰; (F-G) with salinity of 70‰. The Pt peak in the EDS spectra came from sampling coating.

**Figure 10.** SEM images and SEM-EDS composition show the solid products in the solutions with 30 mM citric acid and salinities of 35‰ (A-B) or 70‰ (C-D).
Figure 11. TEM micrographs, EDS composition and SAED pattern for minerals obtained from the system amended with 30 mM succinic acid: (A-C) aragonite; (D-G) Mg-calcite.

Figure 12. TEM micrographs, EDS composition and SAED pattern for minerals obtained from the system amended with 30 mM citric acid: (A) aggregates of disordered dolomite spheroid; (B) disordered dolomite spheroid consisting of many nanocrystallites; (C) a high-resolution image of disordered dolomite displaying a 0.290 nm lattice fringe, corresponding to d-sapcing of (104). The Cu signal in EDS spectrum is attributed to the copper mesh for TEM.
Figure 1

Figure 2
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Figure 6

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Figure 8
Figure 9
Figure 11

Figure 12