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PRECIPITATION OF SYNTHETIC DOLOMITE AT LOW TEMPEARURES WITH
THE INFLUENCE OF MICROBES

by

Sreeram Jambulapati
Bachelor of Science, University of Houston, 2015

A Thesis

Submitted to the Graduate Faculty

Of the

University of North Dakota

In partial fulfillment of the requirements

For the degree of

Master of Science

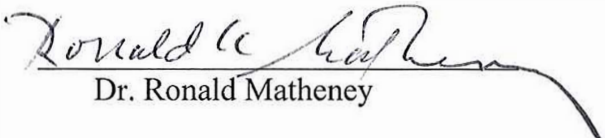
Grand Forks, North Dakota
November
2017

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This thesis submitted by Sreeram Jambulapati in partial fulfillment of the requirements for the Degree of Master of Science in Geology from the University of North Dakota, and has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.



Dr. Stephan Nordeng, Chairperson

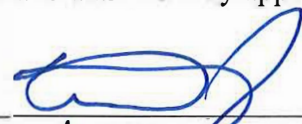


Dr. Ronald Matheny



Dr. Dongmei Wang

This thesis is being submitted by the appointed advisory committee as having met all the requirements of the School of Graduate Studies at the University of North Dakota and hereby approved.



Grant McGimpsey
Dean of the School of Graduate Studies



Date

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Title Precipitation of Synthetic Dolomite at Low Temperatures with the
 Influence of Microbes

Department Harold Hamm School of Geology and Geological Engineering

Degree Master of Science

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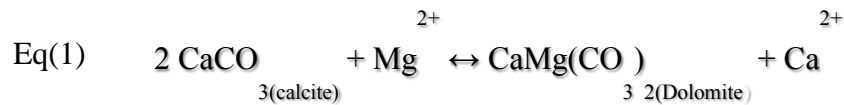
ABSTRACT

New approaches in carbonate geochemistry are aiding geoscientists in understanding dolomite formation. Dolomite is largely absent in modern depositional environments but is present in ancient rocks. The current problem is the inability to synthesize it under low-temperature conditions in the laboratory. Until recently laboratory preparation of dolomite required elevated temperatures (<100°C) to overcome kinetic barriers. One novel approach involves using bacteria to overcome the kinetic barriers that have frustrated efforts to synthesize dolomite at near surface temperatures. This study presents preliminary results of experiments in which three strains of bacteria were used to inoculate a magnesium deficient amorphous calcium carbonate (Mg-ACC) medium (CO_3^{2-} : Mg^{2+} : Ca^{2+} in 2:1:1 ratio). The bacteria used include an aerobic strain, *Virgibacillus marismortui* (ATCC® 700626™) and two anaerobic strains, *Desulfovibrio desulfuricans subsp. desulfuricans* (ATCC®29577™) and *Escherichia coli*. All the experiments were conducted at 30°C and 37°C for 40 days. Preliminary XRD results are consistent with the precipitation of a carbonate phase with a dolomite-like XRD peak near 31° 2θ, Cu $\text{k}\alpha$ radiation. Similar peaks were not apparent for the experiments using the aerobic strain nor the bacteria free control. These results indicate that the two strains of anaerobic bacteria may aid in the formation of a magnesium-rich carbonate phase similar to dolomite at low temperature, within short periods of time.

CHAPTER I
INTRODUCTION

Background

Science changes over time with new methods that result in new experiments. The “Dolomite Problem” is considered an enigma in the field of sedimentary geology and has continued for the better part of two centuries. Research involving microbes to precipitate carbonates is a new approach in understanding formation of dolomite. The origins and method of dolomite formation is debated. Dolomite is found throughout the geologic record, as far back as the Precambrian, with thick units in bulk limestone across the world (Ries 2011). The complexity of this mineral’s formation is that it should form directly from solutions containing magnesium, calcium and carbonate ions (Sadooni 2009). When exposed to magnesium rich fluid, metastable carbonates exchange calcium ions for magnesium ions making a stable carbonate which is dolomite (Sadooni 2009).



Dolomite eq(1), is thermodynamically the most stable carbonate phase in seawater. Dolomite could form as a primary precipitate or a diagenetic replacement with sufficient magnesium source and saline environment with moving fluid but does not form in the present. It can form in different waters such as sea water and waters where brines

mix with ocean water (Warren 2000). In anoxic reducing conditions, bacteria can act to facilitate the primary precipitation of dolomite (Warren 2000). Dolomite frequently recrystallizes after initially forming which is metastable, disordered and non-stoichiometric (Nordeng 1993). Many dolomites have crystals that have no trace of their precursors. This is because nonstoichiometric dolomite crystals can be replaced by more stable phases later (Braithwaite 1991).

The chemistry and kinetic barriers are parameters that have been identified as reasons for much debate and uncertainty. One of the interesting interdisciplinary ideas to come forth is microbial mediation. The prominent kinetic inhibiting factors are thought to involve the high hydration energy of magnesium, low activity of carbonate ions and or low concentrations of sulfate (Rao 2003). Due to ambiguities in reaction kinetics, the mineral's origins have been linked to inorganic chemical models like seepage reflux of concentrated brines or dolomite oversaturation caused by fresh and seawater circulation. Neither model has been demonstrated in experimental conditions and therefore cannot be reproduced (Mckenzie 2010). Geochemical models have lately included a microbiological approach that is now gradually being recognized. In this model, microbes, under specific conditions appear to mediate the formation of dolomite at low temperature (Mckenzie 2010).

Carbonate diagenesis through biological means can certainly garner interest and new information as well as perspective. Including a biogenic approach can bring new perspective to biominerals in carbonate research. (Mckenzie 2010). While there is research showing microbial precipitation of dolomite, it is a stepping stone in answering

the enigma of dolomite formation. Research demonstrating biogenic origins of dolomite may describe biological mechanisms for biomineralization (Nash 2011). Applying laboratory techniques from molecular biology, in testing dolomite would help in understanding the role that microbial metabolism plays in facilitating precipitation (Mckenzie 2010). To mineralize, a localized zone must maintain super saturation (Vasconcelos 2009). Biominerals like pyrite and biogenic calcite have spherical structures linking to microbial activity (Vasconcelos 2009).

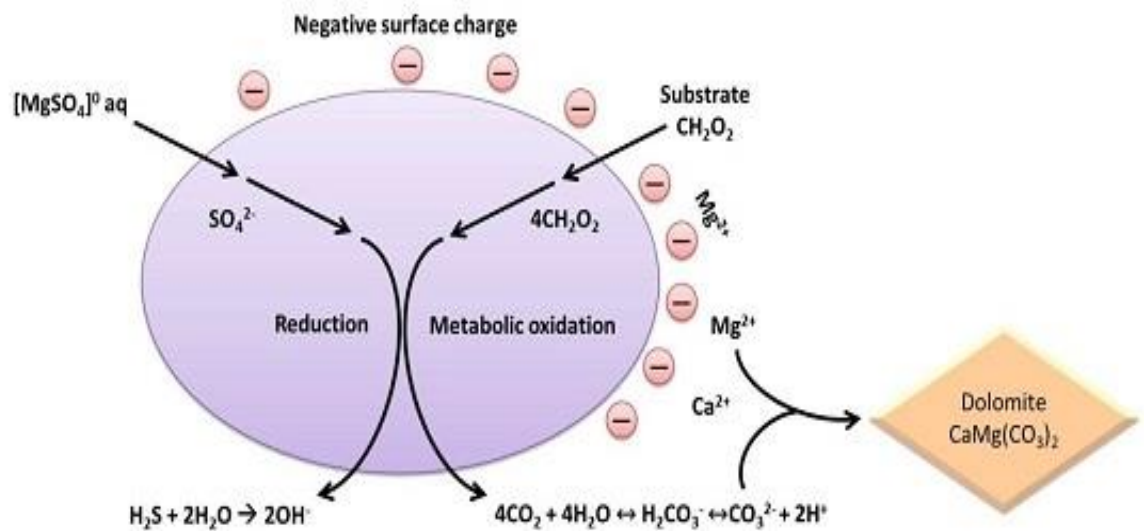


Figure 1. The microbial dolomite model, showing mechanism of precipitation. (Vasconcelos 2008)

The microbial dolomite model (Fig 1) shows the bacterial schematic for the precipitation of dolomite. This is the model that the experiment is based on and tests three different bacteria. According to the microbial model, cell walls acts as nucleation sites, from which, mineralized globules detach from the surface and leave the bacterial cell

intact (Vasconcelos 2009). Precipitation can steadily occur in an organic matrix and microbes with different metabolic pathways promote dolomite formation (Mckenzie 2010). The motivation in this experiment is to replicate the microbial mediation of dolomite formation. In addition, this is an interesting combining of a geological problem with microbiology and molecular biology.

Previous Works

From algae, aerobic, aero-tolerant anaerobic to aero sensitive sulfate reducing bacteria, each classification of bacteria has different metabolic pathways and requirements for growth. Understanding biological constraint of geological problems can offer solutions through experiments (Vasconcelos 2009). Cultured experiments using isolated sulfate reducing bacteria and precipitating dolomite at low temperatures have been conducted (Vasconcelos 2009). The microbial dolomite model developed from experiments in laboratory that precipitated dolomite under aerobic as well as anaerobic conditions (Vasconcelos 2009). This study demonstrated microbial metabolism may control conditions that overcome kinetic barriers (Vasconcelos 2009).

Another study testing mixing zone with sulfate reducing bacteria to promote a dolomitizing environment was conducted. Sumrall (2014) showed that microbes can change their environment by exchanging ions and producing products that would cause a change in concentrations of calcium and magnesium. The study suggested that microbes act as sites of nucleation for carbonate crystallites (Sumrall 2014).

In China, a similar experimental design was used to test microbial dolomite precipitation. A core from Qinghai Lake, and the isolated sulfate reducing bacteria for

their analysis were obtained. This study concluded that overcoming the kinetic barrier and dolomite precipitation was due to the increase in pH, removal of sulfate and carbonate alkalinity (Deng 2010). In the Coorong region of southwest Australia, a similar study was carried out. In this study, the lake sediments and bacteria were available from the lake being studied. They precipitated dolomite and concluded that sulfate reducing bacteria overcame kinetic barriers. This was done in hypersaline solution, by removing sulfate, releasing calcium and magnesium ions and increasing carbonate ion concentration (Wright 2005). In Brazil, water samples and the bacterial were collected and isolated from the study location of Lagoa Vermelha, a hypersaline lagoon.

The study was successful in that dolomite was precipitated with a consistent dumbbell morphology, similar to the shape in the observed lake sediments (Lith 2000). An experiment using aerobic bacteria, *Virgibacillus marismortui* (ATCC® 700626™), was able to show precipitation of dolomite within 30 days of incubation at 25-30°C, also showing that precipitation decreased with elevated temperatures and increases with more incubation time (Banerjee 2016).

The inspiration for using a precursor carbonate in this experiment was taken from Rodriguez-Blanco (2015) The precursor is a magnesium deficient amorphous calcium carbonate (Mg-ACC). The experiment they conducted involved dehydrating the Mg-ACC that caused it to nucleate into a non-stoichiometric proto dolomite. After some time on a larger scale of days, the disordered proto dolomite transformed into ordered and stoichiometric dolomite (Rodriguez-Blanco 2015).

In a magnesium free ACC, the precursor converted into vaterite by spherulitic growth and then gradually into calcite via dissolution and re precipitation (Rodriguez-Blanco 2015). The growth was shown to occur due to a nucleation mechanism and the growth of vaterite from the ACC was comparable to the dolomite formed via microbial metabolism (Rodriguez-Blanco 2015). They further speculate that microbial carbonates may have also required such a precursor to facilitate spherulitic growth (Rodriguez-Blanco 2015). Experiments demonstrating the microbial model have been conducted in Australia, China, India and Brazil by collecting samples of dolomitized sediment and tested with bacterial cultures isolated from the location.

This experiment will try to accomplish the same but under strict laboratory conditions using bacteria and an Mg-ACC precursor. It is hypothesized that proto dolomite can be synthesized by simply adding microbes to a magnesium deficient amorphous calcium carbonate (Mg-ACC) media to form ordered stable dolomite.

CHAPTER II

EXPERIMENTAL METHODS

The experiment is a geomicrobiological approach to enabling growth of solid crystals in an aqueous solution. Precipitating dolomite using the influence of microbes at low temperatures is the objective. Two bacterial strains were ordered from ATCC (The Global Bioresource Center), *Virgibacillus marsimortui* (ATCC® 700626™) and *Desulfovibrio desulfuricans subsp. desulfuricans* (ATCC®29577™). In addition to these strains, *Escherichia coli* was also used which is a facultative anaerobe. The ordered strains are halophilic aerobic and sulfate reducing anaerobic respectively. Additionally, each strain has its own metabolic properties which may provide insight into how kinetic inhibition is overcome. Each strain in the vials tested was given a number for identification.

Culturing *Escherichia coli*, *Virgibacillus marismortui* (ATCC® 700626™) and *Desulfovibrio desulfuricans subsp. desulfuricans* (ATCC®29577™) was done by following instructions provided by the ATCC. The tip of the vial is broken by heating it and then adding a few drops of cold water to crack the vial tip. The vials contained a secured freeze dried powder at the bottom which is to be rehydrated carefully without contamination. The bacterial strains are handled separately based on nature and media. The media in which both bacterial strains are to be grown were clearly stated in the

documentation sheet provided by ATCC. For the halophilic aerobic bacteria, the vial is opened and cautiously rehydrated with the prepared media. All the chemicals, their quantities, and the method are noted and carried out in order per instructions (Appendix A). To grow the anaerobic bacterial strain, the contents of the vial is examined first to check for compromise. Autoclaving the media in which it is to grow follows after making the media by carefully adding the specified amounts of chemicals mentioned in the provided media list (Appendix A)..

When handling the chemicals, each component was measured, and the solution was adjusted to the corresponding pH (Appendix A). Once the solution was adjusted, it was autoclaved. After the autoclaving process, a precipitate formed at the bottom which was removed by filtering the media solution through a 2-micron filter. Carefully, a little of the media was added to the bacterial vial to rehydrate and then placed back in the solution vial to grow.

The amorphous magnesium deficient calcium carbonate is an important component of the experiment because it is an approach to determine if a calcium rich semi solid can transform into a solid mediated by microbial metabolism. The amorphous calcium carbonate is made separately, at room temperature by mixing the required chemicals (Appendix A). Upon reacting, a white gel is instantaneously precipitated and is inoculated with a bacterial strain and its respective growth media. The media is set aside and left to nucleate. The controls in this experiment include two vials for each bacterial strain. One vial containing only the growth media, the second with only the bacteria and its growth media. This was to check for any dolomitization that might occur in the

absence of combining the amorphous carbonate. A third vial for each strain with only the media and amorphous carbonate was made a control for testing precipitation without a bacterial strain. The fourth vial for each strain was a mixture of the strain, growth media and amorphous carbonate.

Evaluation of Precipitates

(XRD) or X-ray diffraction analysis was used for phase identification of a crystalline structure can provide information about unit cell dimensions. To run the analysis, a sample was collected from the bottom of each vial containing the amorphous carbonate, dried by heating in a crucible to a fine powder and placed onto a specimen holder in the XRD using Cu α radiation. Using Bragg's Law, defined by $n\lambda=2d\sin\theta$, peaks can be found on X-ray pattern at θ values that correspond to each of the d-spacings that characterize the mineral of interest. In the equation, n is the order, λ is a wavelength, d is the lattice spacing and θ is the crystal orientation.

The run time for each sample was roughly 40 minutes to 90 minutes depending on the length of the 2θ scale of interest. For some samples, the scale was from 10 to 60 degrees 2θ , run at half degree per minute and for others it was from 20 to 40 degrees 2θ , run at one degree per minute. The other method is (SEM) or scanning electron microscope, which will create an image by producing signals that can provide information about surface topography as well as something about the composition. In the SEM, a small sample is placed onto a carbon conductive adhesive tab and examined at powerful **MAGNIFICATION** to see the morphologies in the sample. For the SEM analysis,

only the vials that showed positive results for carbonates in the XRD scan were investigated further to see the budding mechanism of precipitation.

Table 1. The Bacterial Strains and the Experimental Controls Used are Presented in the Table Showing how the Vials were going to be Inoculated.

Bacterial Strains	Media only	Strain and media	Media and (Mg-ACC)	Strain with media and (Mg-ACC)
<i>Escherichia coli</i>	5ml	5ml	4 ml media+ 1ml amorphous (Mg-ACC)	4 ml media+ 1ml amorphous (Mg-ACC)
<i>Virgibacillus marismortui</i> (ATCC® 700626™)	5ml	5ml	4 ml media+ 1ml amorphous (Mg-ACC)	4 ml media+ 1ml amorphous (Mg-ACC)
<i>Desulfovibrio desulfuricans</i> subsp. <i>desulfuricans</i> (ATCC® 29577™)	5ml	5ml	4 ml media+ 1ml amorphous (Mg-ACC)	4 ml media+ 1ml amorphous (Mg-ACC)

CHAPTER III

RESULTS

After the amorphous carbonate was incubated and left in the vials for 40 days, there were positive results for the precipitation of solid carbonates. There was a mixture of calcite and or magnesite present within almost all the vials with some dolomite in small amounts, some showing faint peaks. XRD analysis was interpreted using standard 2θ peak data from the American mineralogist crystal structure database, with which peaks from the sample vials were compared (Appendix C). Standard peaks for calcite, dolomite, magnesite and vaterite were selected and compared to peaks from each vial and matched based on XRD scans. SEM analysis was conducted by marking points of potential carbonate crystals and magnified at 10 micrometers. From (Appendix C) every strain used was able to precipitate dolomite based on the XRD peaks. Figures 1, 4, 7 and 11, show labeled peaks for dolomite on the XRD scans. The SEM analysis shows calcite crystals for *Escherichia coli* as well as stoichiometric percentages for. Tables 2 and 3 shows the strains with their respective labelled vials and shows results of carbonate precipitation based on XRD and SEM.

Table 2. Experimental Numbered Vials with the Strains used showing Precipitation of the Types of Carbonates based on XRD and SEM Analysis. (Bold numbers represent Vials name)

Results Based On	XRD,	SEM	XRD	XRD
Experimental Vials	Calcite Precipitation	Calcite Precipitation	Magnesite Precipitation	Dolomite Precipitation
ATCC Medium 1249 and amorphous carbonate (1)	Yes (Fig 11)	Yes (Fig 26)	Yes	Yes (Fig 1)
ATCC Medium 1249 with <i>Desulfovibrio desulfuricans</i> subsp. <i>desulfuricans</i> (ATCC®29577™) and amorphous carbonate (2)	No (Fig 7)	Yes (Fig 23)	No (Fig 7)	Yes (Fig 7)
ATCC Medium 2101 and amorphous carbonate (3)	No (Fig 3)	-	Yes (Fig 3)	No (Fig 3)
ATCC Medium 2101 with <i>Virgibacillus marismortui</i> (ATCC® 700626™) and amorphous carbonate (4)	No (Fig 2)	-	Yes (Fig 2)	Yes (Fig 2)
<i>Luria-Bertani</i> Media and amorphous carbonate (5)	Yes (Fig 6)	-	Yes (Fig 6)	No (Fig 6)
<i>Luria-Bertani</i> Media with <i>Escherichia coli</i> and amorphous carbonate (6)	Yes (Fig 4)	Yes (Fig 5)	Yes (Fig 4)	Yes (Fig 4)

Table 3. The Table Shows no Activity or Precipitation in the Control Vials with only Media and Bacteria and Media Only.

Experimental Vials	Precipitation in Control Vials
<i>Escherichia coli</i> (*)	No
<i>Virgibacillus marismortui</i> (ATCC® 700626™) (*)	No
<i>Desulfovibrio desulfuricans</i> subsp. <i>desulfuricans</i> (ATCC®29577™) (*)	No

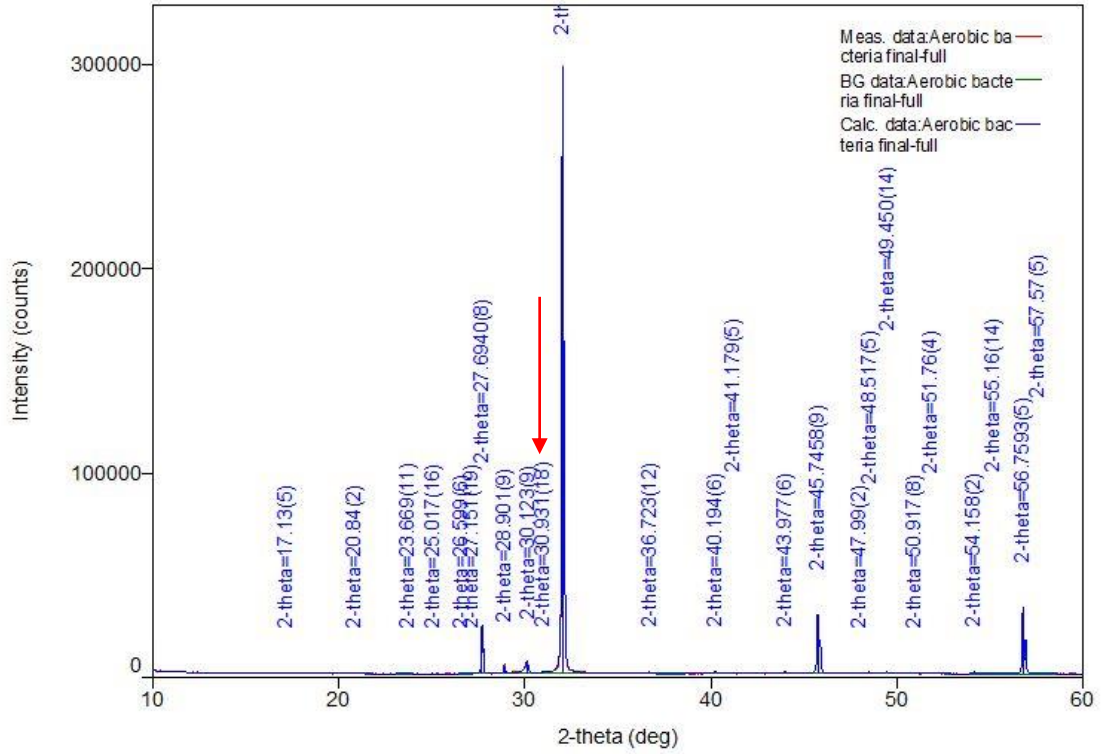


Figure 2. ATCC® Medium 2101: *Bacillus marismortui*, *Virgibacillus marismortui* (ATCC® 700626™) and amorphous carbonate. In this image, XRD scan shows very weak signal for dolomite as shown by the arrow. The figure was magnified to show the peak (Appendix B Figure 17).

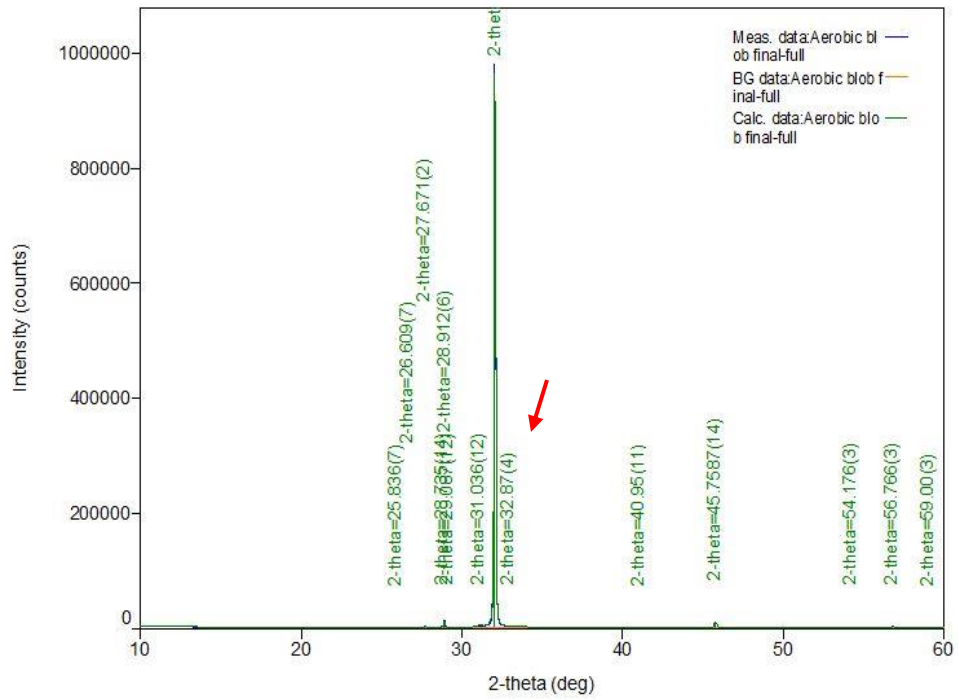


Figure 3. ATCC® Medium 2101: *Bacillus marismortui* and amorphous carbonate. In the figure, XRD scan shows no signal for dolomite and weak signal for magnesite (red arrow).

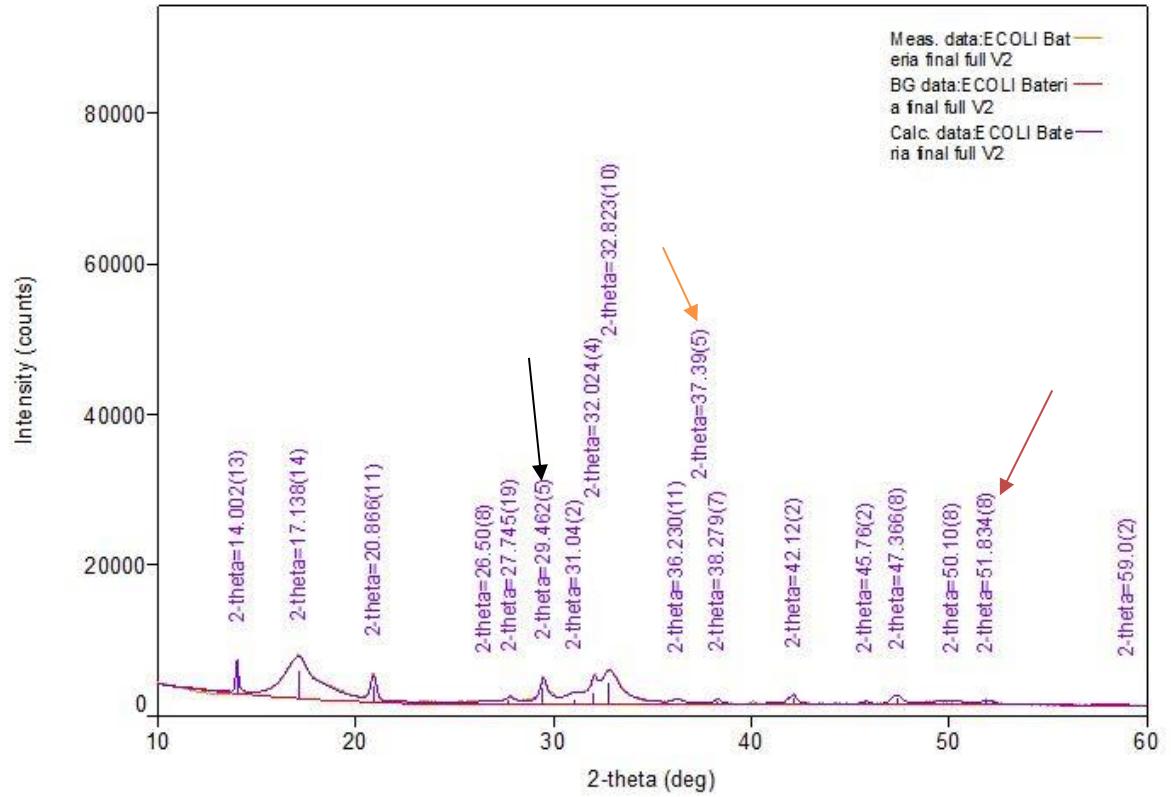


Figure 4. *Escherichia coli*, Luria-Bertani media and amorphous carbonate. In the figure above, there was no signal for dolomite. However, there was signal for precipitation of magnesite (red arrow), a faint peak for dolomite (orange arrow) and calcite (black arrow). The figure was magnified to show the peak (Appendix B Figure 20).

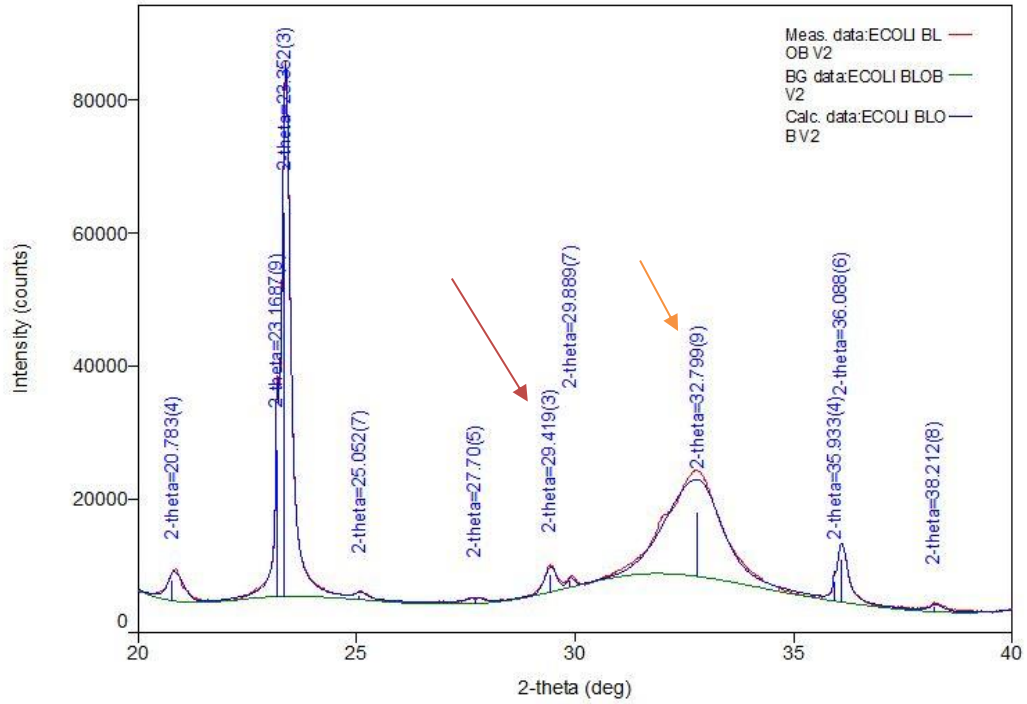
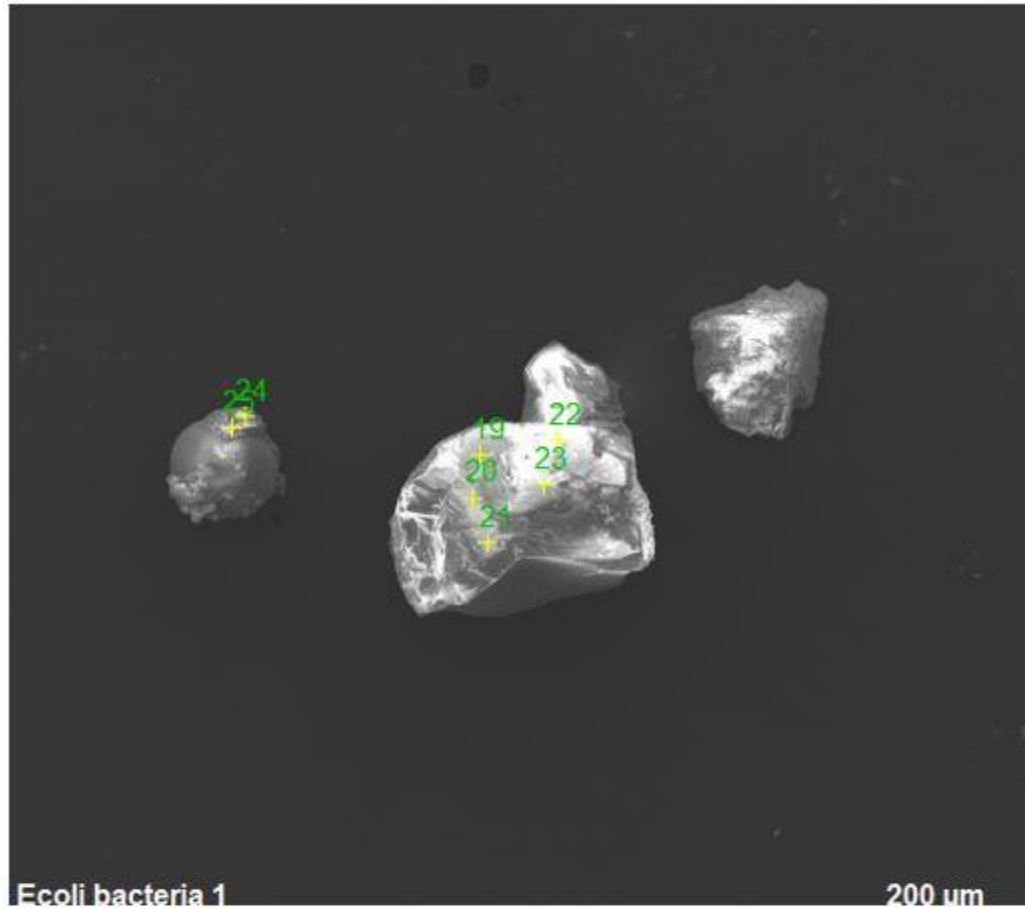


Figure 5. *Luria-Bertani* media and amorphous carbonate. In the above figure, there is a peak for calcite (red arrow) and magnesite (orange arrow). There was no peak for dolomite. The figure was magnified to show the peak (Appendix B Figure 30).



Norm. mass percent (%)

Spectrum	C	O	Na	Mg	Si	P	Cl	Ca
24	17.68	41.56	4.38	10.17	2.65	1.12	4.15	18.30
25	21.68	17.42	25.86	2.61	-	0.71	29.30	2.43
Mean value:	19.68	29.49	15.12	6.39	2.65	0.91	16.72	10.36
Sigma:	2.83	17.07	15.19	5.34	0.00	0.29	17.78	11.22
Sigma mean:	2.00	12.07	10.74	3.78	0.00	0.21	12.58	7.93

Figure 6. SEM analysis of *Escherichia coli*, Luria-Bertani media and amorphous carbonate.

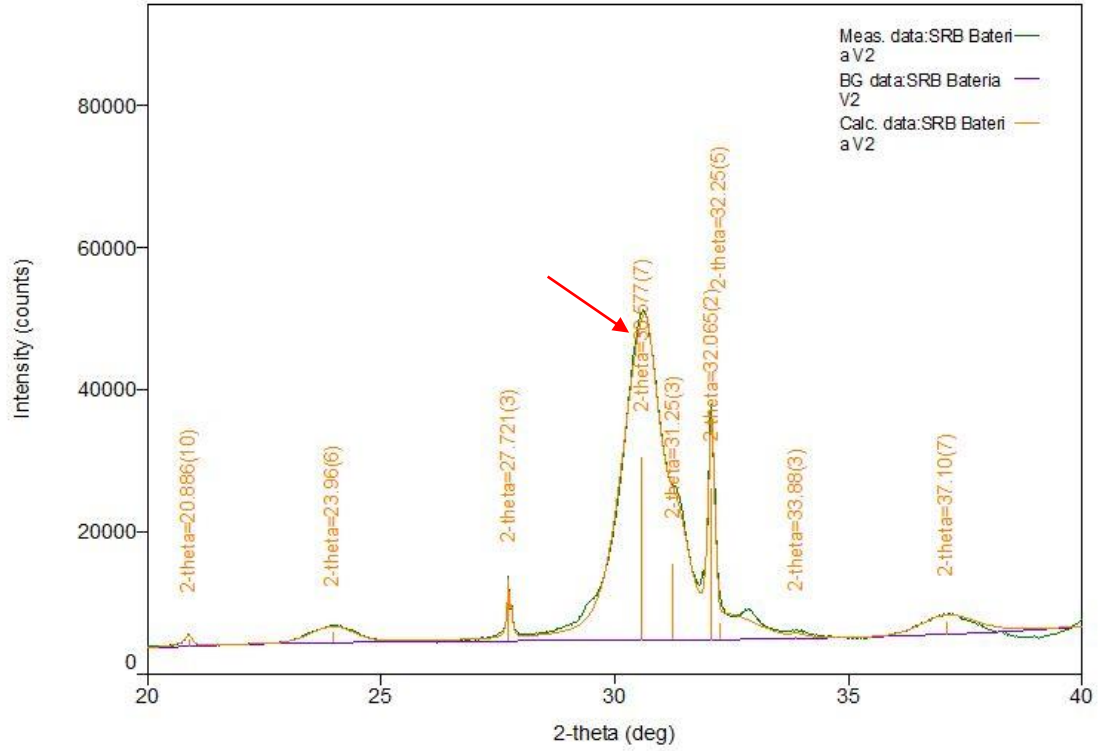


Figure 7. ATCC Medium 1249: Modified Baar's media, *Desulfovibrio desulfuricans* subsp. *desulfuricans* (ATCC®29577™) and amorphous carbonate. In the above figure, there is a signal for dolomite (red arrow).

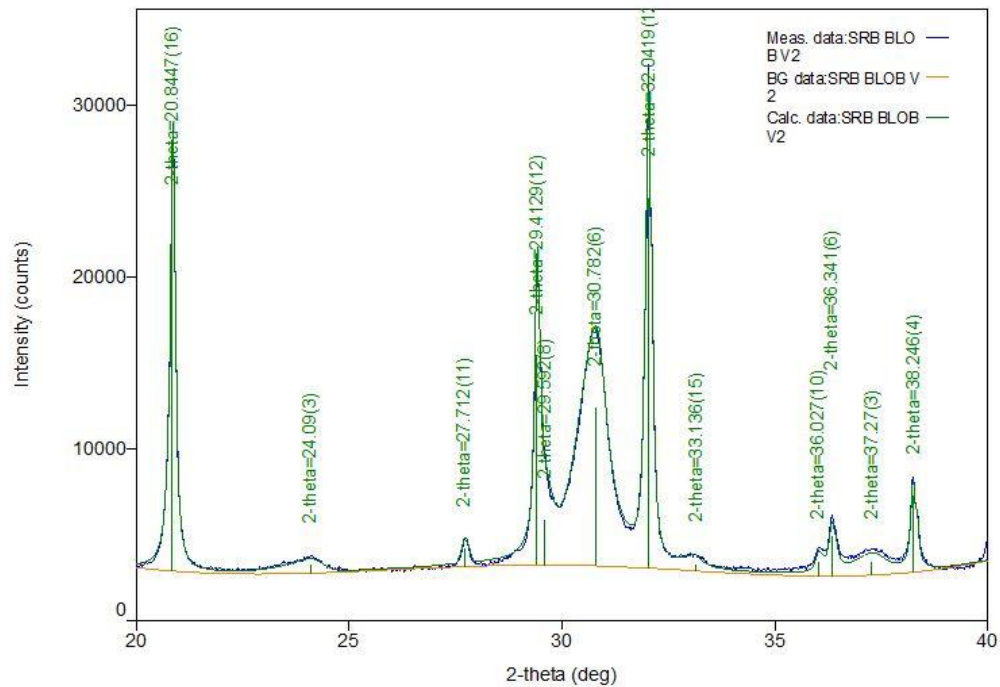


Figure 8. ATCC Medium 1249: Modified Baar's media and amorphous carbonate only. In the above figure, there is a signal for dolomite (red arrow).

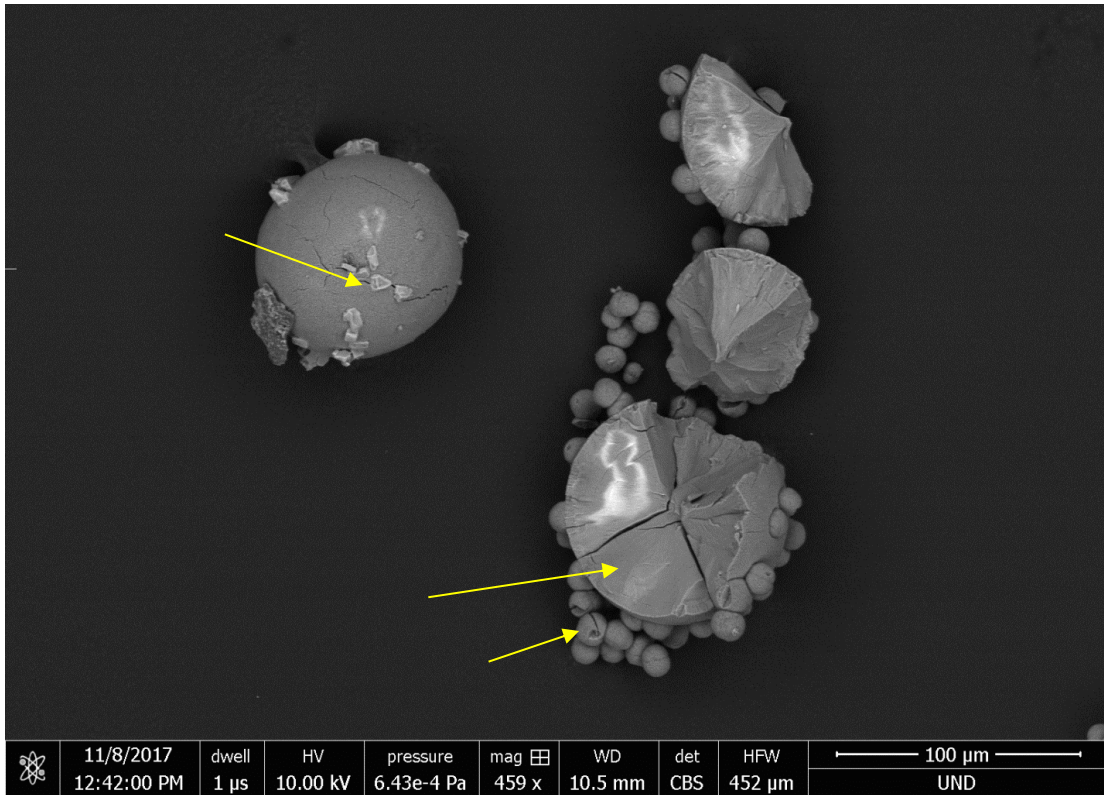
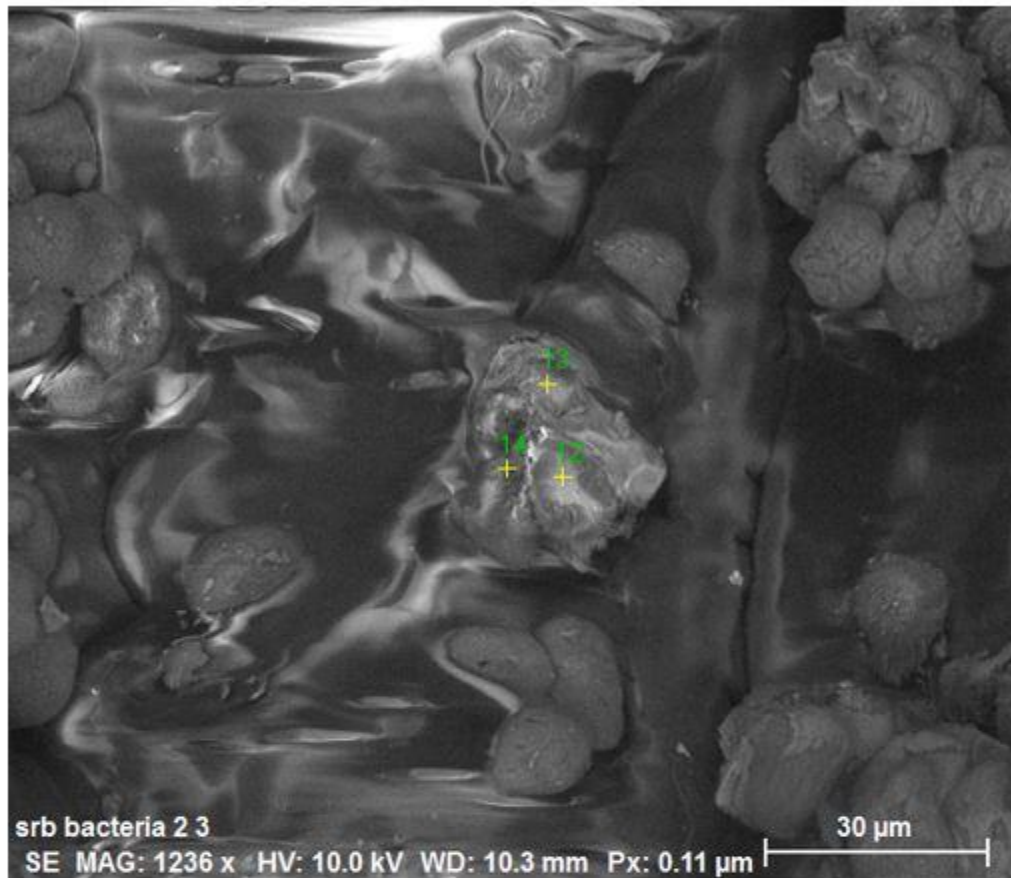


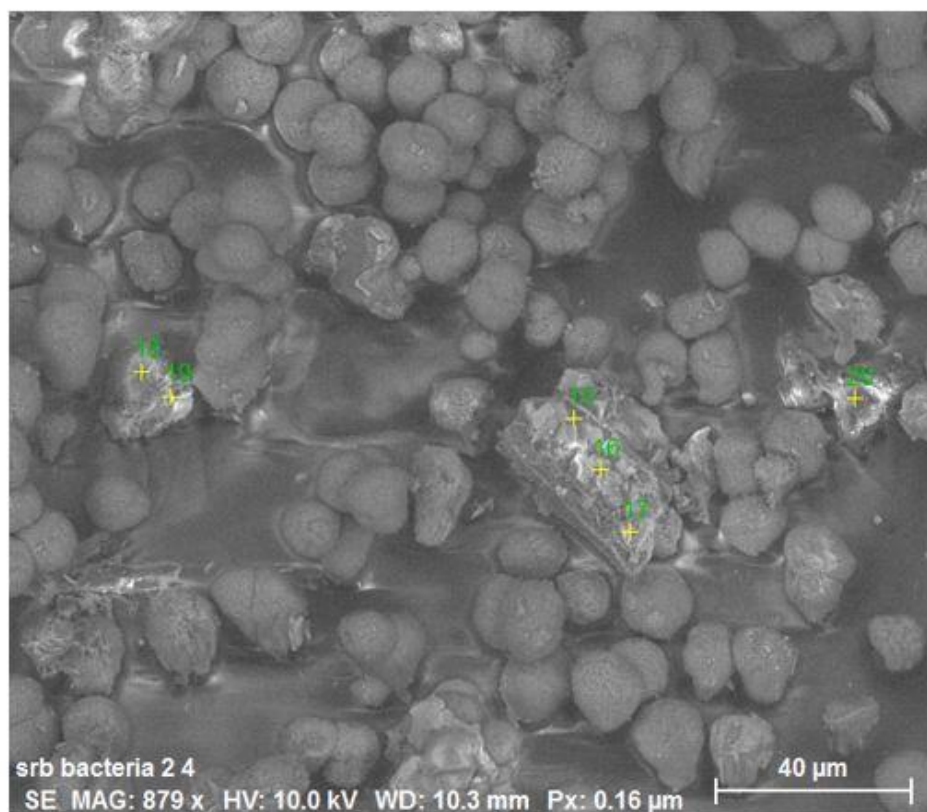
Figure 9. ATCC Medium 1249: Modified Baar's media, *Desulfovibrio desulfuricans* *subsp. desulfuricans* (ATCC®29577™) and amorphous carbonate. In the figure above, there appears small partial rhombohedral crystals budding off the bacteria which could be dolomitic in composition.



Norm. mass percent (%)

Spectrum	C	O	Na	Mg	P	S	Cl	K	Ca
12	11.90	40.52	7.74	6.40	-	-	11.33	-	22.10
13	27.75	49.21	4.94	8.68	0.30	0.72	0.69	-	7.71
14	49.97	24.58	6.07	2.33	-	2.18	8.81	1.17	4.89
Mean value:	29.87	38.11	6.25	5.81	0.30	1.45	6.94	1.17	11.56
Sigma:	19.12	12.49	1.41	3.22	0.00	1.03	5.56	0.00	9.23
Sigma mean:	11.04	7.21	0.81	1.86	0.00	0.60	3.21	0.00	5.33

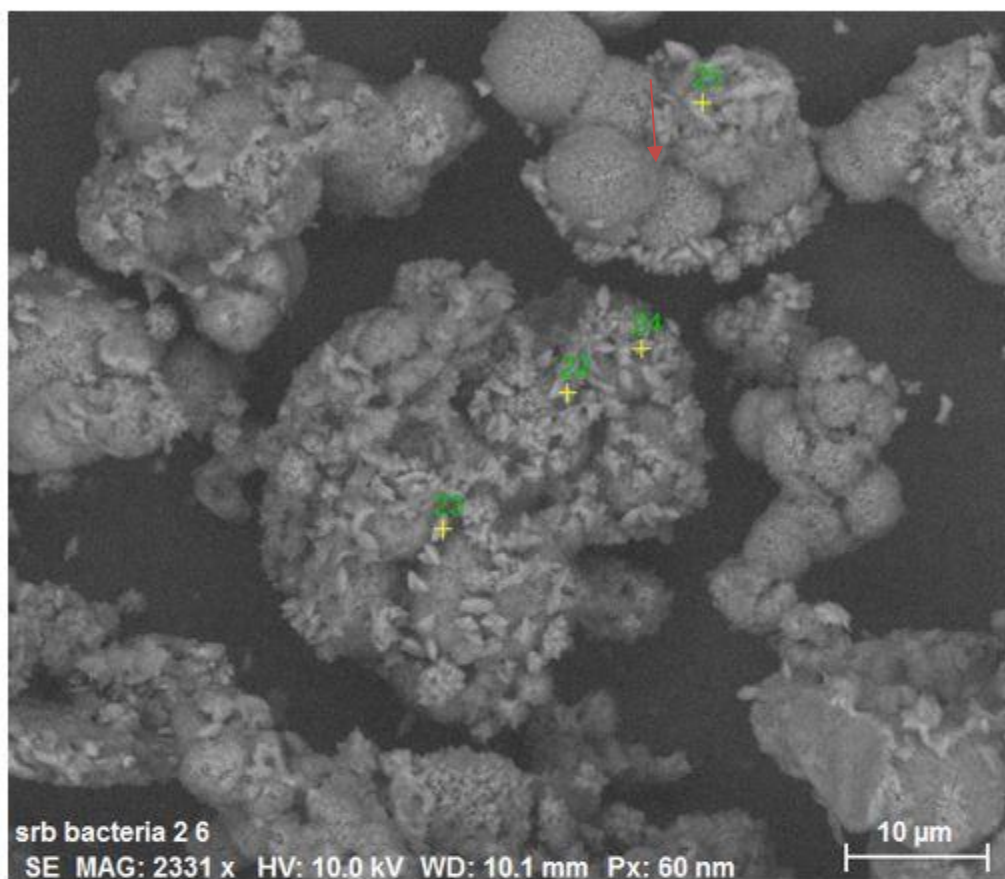
Figure 10. ATCC Medium 1249: Modified Baar's media, *Desulfovibrio desulfuricans subsp. desulfuricans* (ATCC®29577™) and amorphous carbonate. The above figure shows SEM analysis of several points



Norm. mass percent (%)

Spectrum	C	O	F	Na	Mg	Al	Si	P	S	Cl	Ca
15	16.17	18.41	-	29.67	2.04	-	-	-	0.76	32.96	-
16	20.48	33.74	-	19.72	8.01	-	-	-	0.79	17.26	-
17	47.00	14.48	-	5.32	3.85	-	-	-	-	24.93	4.42
18	21.70	48.20	-	0.68	6.74	-	0.55	0.47	0.30	2.20	19.16
19	22.72	8.52	-	0.17	0.55	-	-	-	-	60.46	7.59
20	52.91	34.43	0.21	-	-	8.26	3.30	-	-	0.55	0.33
Mean value:	30.16	26.30	0.21	11.11	4.24	8.26	1.92	0.47	0.62	23.06	7.88
Sigma:	15.60	14.96	0.00	13.04	3.13	0.00	1.94	0.00	0.27	22.25	8.09
Sigma mean:	6.37	6.11	0.00	5.32	1.28	0.00	0.79	0.00	0.11	9.08	3.30

Figure 11. ATCC Medium 1249: Modified Baar's media, *Desulfovibrio desulfuricans* subsp. *desulfuricans* (ATCC®29577™) and amorphous carbonate. The above figure shows SEM analysis of several points.



Norm. mass percent (%)

Spectrum	C	O	Na	Mg	P	S	Cl	K	Ca
22	13.14	57.06	1.86	2.17	0.53	-	1.12	-	24.12
23	9.66	45.49	1.71	2.49	0.94	-	2.02	-	37.70
24	12.21	53.30	1.98	2.86	0.80	-	1.33	-	27.51
25	35.41	26.14	14.72	3.46	-	1.22	14.44	0.34	4.26
Mean value:	17.61	45.50	5.07	2.75	0.75	1.22	4.73	0.34	23.40
Sigma:	11.96	13.78	6.44	0.56	0.21	0.00	6.49	0.00	14.00
Sigma mean:	5.98	6.89	3.22	0.28	0.10	0.00	3.24	0.00	7.00

Figure 12. *Desulfovibrio desulfuricans* subsp. *desulfuricans* (ATCC®29577™) with media and amorphous carbonate only.

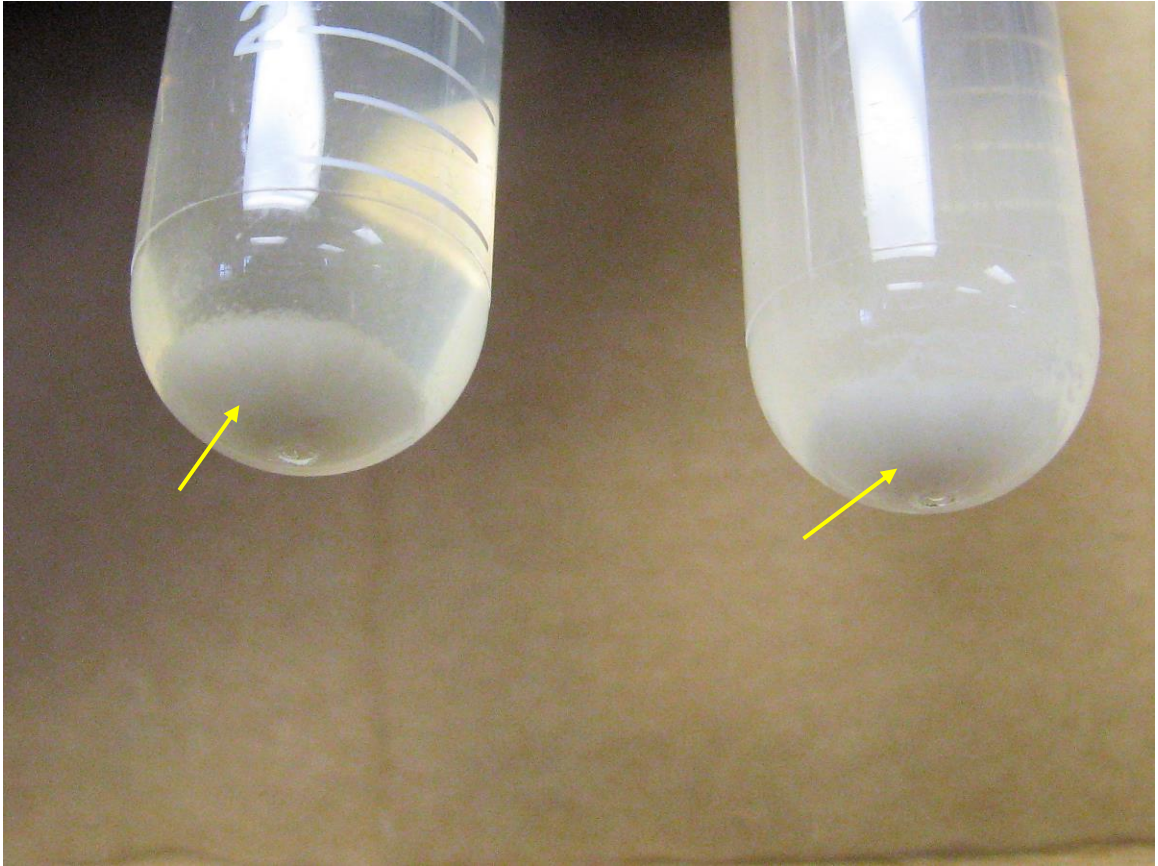


Figure 13. Experimental vials for *Desulfovibrio desulfuricans subsp. desulfuricans* (ATCC®29577™) showing white precipitate (yellow arrow) at the bottom.

CHAPTER IV

DISCUSSION

Based on the experiment with three bacterial strains used, the results suggest that there is a biological component to precipitating carbonates, especially dolomite. Using the XRD and SEM analysis, carbonates such as calcite, magnesite and dolomite precipitated in 40 days using an amorphous carbonate, bacteria and growth media. The morphology and composition of the produced carbonates were observed in the SEM. Whether proto dolomite could be synthesized by microbes at low temperature to accelerate Mg-ACC to ordered stable dolomite, was the subject of the experiment.

Based on the previous studies and this experiment, the microbial model appears valid. The idea for the amorphous carbonate was taken from (Rodriguez-Blanco 2015), in which mixing chemical components would create a carbonate pre cursor. This method had not been conducted before which should be interesting to see, whether there is growth of dolomite if anything at all. Similar experiments in which a solid dolomitic mass was precipitated by microbes has been well documented, however, this is still a new methodology in sedimentary geology. The difference between previous experiments conducted was that dolomite, water samples and the isolated bacterial culture were taken from the study location.

In contrast, this experiment attempted to produce dolomite from scratch in a lab, using an amorphous carbonate as opposed to readily available carbonate samples. This would allow microbes to use chemicals from the amorphous component to carry out their metabolism. The idea from here is that carbonates precipitation would be a result of a biological reaction which would bypass a kinetic inhibition that prevents precipitation. The premise that dolomite may be a bio mineral was also suggested by (Vasconcelos 2008) paper in which the microbial model was broken down and explained through a geo-microbiological perspective.

From figure 14, taken from (Goldsmith 1961), the dolomite 2θ peak values were plotted to show relative molar percent of magnesium. As the percentage of magnesium increases, the c-axis contracts producing the d spacing along the 104 plane.

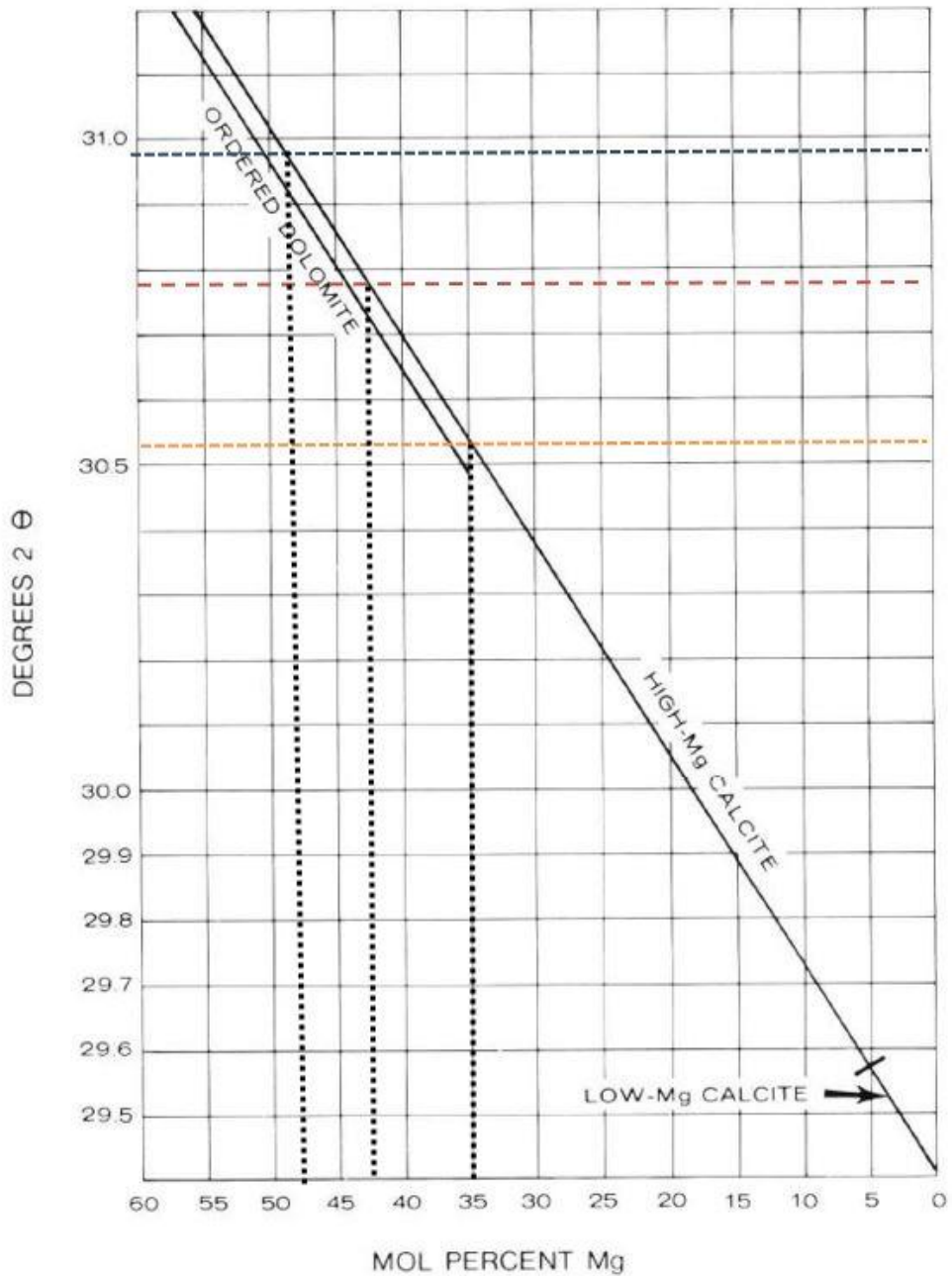


Figure 14. The graph displays where XRD analysis shows dolomite peaks and a dashed solid line to show approximate mol percent of magnesium. The orange and red lines represents the *Desulfovibrio desulfuricans subsp. desulfuricans* (ATCC®29577™), with orange (bacteria, amorphous, media) and red (media and amorphous only) respectively. The navy blue line (bacteria, media and amorphous) represents *Virgibacillus marismortui* (ATCC® 700626™).

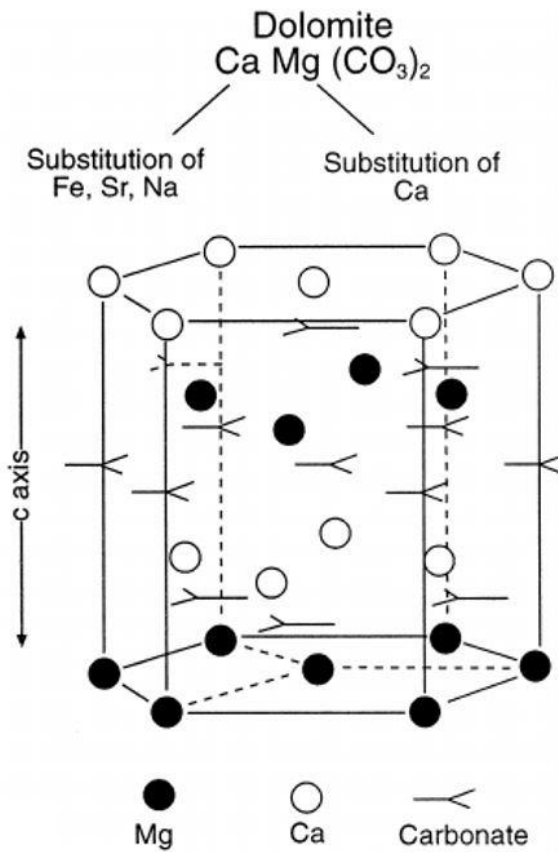


Figure 15. Dolomite structure showing the lattice of calcium, magnesium and carbonate ions and how they organize. (Warren 2000)

For the peaks observed in the XRD scans, the signals for some samples were weak. This could be due to the fact that the sample to work with was insufficient and were not washed thoroughly enough due to potential damage to the crystals present in the sample. The results from *Desulfovibrio desulfuricans subsp. desulfuricans* (ATCC®29577™) suggest the media could be the source of nutrients that overcome kinetic barriers in the precipitation reaction. This experiment is important to show that

there is a geomicrobiological perspective in carbonate precipitation that has been overlooked until recent studies.

To understand this mineral, researching the properties of its formation and kinetics are necessary. With the proper treatment of the media, bacteria and a controlled environment, dolomite crystals can grow and may form a clusters around the bacterial strain. This result would also demonstrate that the microbial model is testable, reproducible and valid. Additionally, the usage of a semi solid pre-cursor would indicate that certain conditions must be met but the range of variables could be wide with respect to temperature and bacterial concentration.

The results show dolomite precipitation by each bacterial strain with its respective growth media and the amorphous carbonate. The ATCC Medium 1249 and amorphous carbonate showed precipitation of dolomite without the presence of bacteria. This could suggest that the media is the organic source for the precipitation while the bacteria may be a component that enhances the process in this case. The other vials with the bacteria showed that the bacteria were required for dolomite to precipitate as the controls showed no signal for dolomite. The ATCC Medium 1249 under anaerobic conditions could be a factor in the mediation of dolomite, in the presence of an amorphous carbonate precursor. In contrast, the Luria-Bertani and ATCC medium 2101 with the pre- cursor carbonate showed no signal in aerobic conditions.

With this microbial model, the bio mineral aspect is gaining recognition as it has been demonstrated by different researchers but not entirely from scratch as this experiment has attempted to do. Based on the results of this experiment, dolomite can be

considered a bio mineral and can be precipitated by microbes at low temperatures in a controlled environment. The question to answer going forward is how and where amorphous carbonate would be present in the nature to provide similar conditions for microbial mediation.

CHAPTER V
CONCLUSION

The microbial dolomite model, based on the results of this experiment support the premise that microbial metabolism is capable of precipitating dolomite and other carbonates. The bacterial strains, with their different metabolisms show that some bacteria work at different rates and temperatures to be able to precipitate carbonates.

APPENDICES

APPENDIX A

STRAINS AND COMPONENTS USED

Strains and components used:

- 1) *Escherichia coli*
- 2) *Virgibacillus marismortui* (ATCC® 700626™)
- 3) *Desulfovibrio desulfuricans subsp. desulfuricans* (ATCC®29577™)
- 4) Amorphous calcium carbonate

Strains 2 and 3 were obtained from the American Type Culture Collection (ATCC) for the experiment. The following strains were cultivated in a molecular biology laboratory along with the media required for their growth. Each of the strains used classify as biosafety level 1. In each case for the preparation, the strains were only handled after making the media first.

1) Luria-Bertani broth:

The *Escherichia coli* strain was obtained from the ATCC and cultivated using an enriched media. This media is called Luria-Bertani, it uses amino acids as its source of carbon for the consumption of the bacteria. This is a commonly used media and permits fast growth. To prepare this broth:

- a) 10 grams of tryptone
- b) 5 grams of yeast extract
- c) 10 grams of NaCl
- d) 1 liter of distilled water
- e)

Adjust the pH to 7 with 1 molar NaOH and autoclave. The mixture was prepared using a weighing scale, magnetic stir plate and bar and the chemicals were ordered and ready to use. Each chemical component was measured and added to the mixture in succession. The readily available strain was cultivated in the laboratory for 4 days. The media and bacteria were inoculated by adding the amount of 50 microliters of the bacteria to the media aseptically.

Sezonov, Guennadi. "Escherichia Coli Physiology in Luria-Bertani Broth[∇]." *Journal of Bacteriology* 189 (2007): n. pag. American Society for Microbiology. Web.

2) *Virgibacillus marismortui* (ATCC[®] 700626TM):

This strain was obtained from the ATCC and required the preparation of ATCC[®] medium 2101: *Bacillus marismortui* medium. This aerobic strain was cultivated for a period between 24 to 48 hours at 37°C. To prepare the medium:

- a) 81 grams of NaCl
- b) 7 grams of MgCl₂
- c) 9.6 grams of MgSO₄
- d) 0.36 grams of CaCl₂
- e) 0.026 grams of NaBr
- f) 5 grams of proteose peptone
- g) 10 grams of yeast extract
- h) 1 gram of glucose
- i) 1 liter of deionized water

Using a weighing machine, each of the ingredients were measured and combined using a beaker and magnetic stir plate. Upon combining the components, the final pH was adjusted to 7 using KOH. It was then autoclaved for an hour. The vial was opened by breaking the sealed glass tip. Using the dried bacteria, it was rehydrated. The rehydrated pellet was aseptically transferred back into the broth table and mixed thoroughly.

3) *Desulfovibrio desulfuricans subsp. desulfuricans* (ATCC®29577™)

This strain was obtained from the ATCC and required the preparation of ATCC medium 1249: Modified Baar's Medium for Sulfate Reducers. This anaerobic strain was cultivated for a period between 7-9 days at 30°C. For this strain, the inoculated broth was immediately placed in an anaerobic chamber in which the oxygen was removed by placing an anaerobic gas pack in the chamber. This pack helps the strain grow. As a control, a vial of the bacteria was observed separately to check for possible contamination before proceeding with the experiment. The ingredients required:

Component I

- a) 2 grams of MgSO₄
- b) 5 grams of Sodium Citrate
- c) 1 gram of CaSO₄ x 2H₂O
- d) 1 grams of NH₄Cl
- e) 400 milliliters of Deionized water

Component II

- a) 0.5 grams of K₂HPO₄
- b) 200 milliliters of Deionized water

Component III

- a) 3.5 grams of Sodium lactate
- b) 1 gram of Yeast extract
- c) 400 milliliters of Deionized water

The components were adjusted to a pH of 7.5 and cultivated. They were mixed aseptically after the media to be used was scaled depending on the quantity to be used in the experiment with the amorphous carbonate. In this experiment, this media was scaled and adjusted for 1 liter.

The media was used to rehydrate the bacteria, aseptically, followed by their placement in the gas chamber with a gas mixture of 80% N₂ – 10% CO₂ – 10% H₂. The chamber was sealed and left for 40 days.

4) Amorphous calcium carbonate

The procedure to create the amorphous component was taken from (Rodriguez-Blanco 2015), and prepared according to the ratio described in the literature. In this experiment, these components were adjusted to 100 milliliters. It was combined by taking each chemical component and using a micro pipette to add and mix thoroughly. They were combined and kept in a ratio of CO₃²⁺: Ca²⁺:Mg²⁺ = 2:1:1 at room temperature. The components:

- a) 1 molar Na₂CO₃ solution
- b) 1 molar CaCl₂ solution
- c) 1 molar MgCl₂ solution

The solutions were made by dissolving molar mass in liter of deionized water. Deionized water is reactive and changes when it is exposed to air. Before contact with air, it has a pH of 7 but upon contact with CO₂ becomes an acidic 5.6.

APPENDIX B
FIGURES AND IMAGES

Figures and Images

Virgibacillus marismortui (ATCC® 700626™)

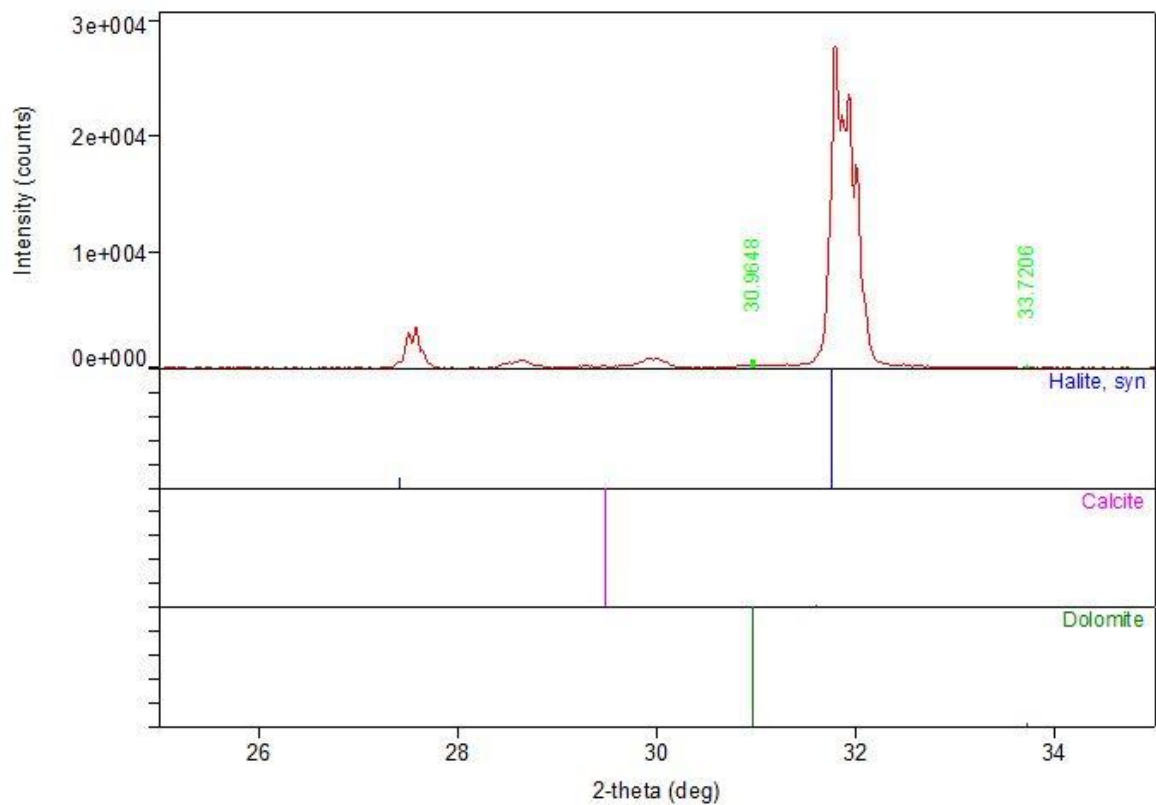


Figure 16. ATCC® Medium 2101: *Bacillus marismortui*, *Virgibacillus marismortui* (ATCC® 700626™) and amorphous carbonate. In the above figure, only the halite peak is apparent. This analysis was done at a small scale and preliminary test.

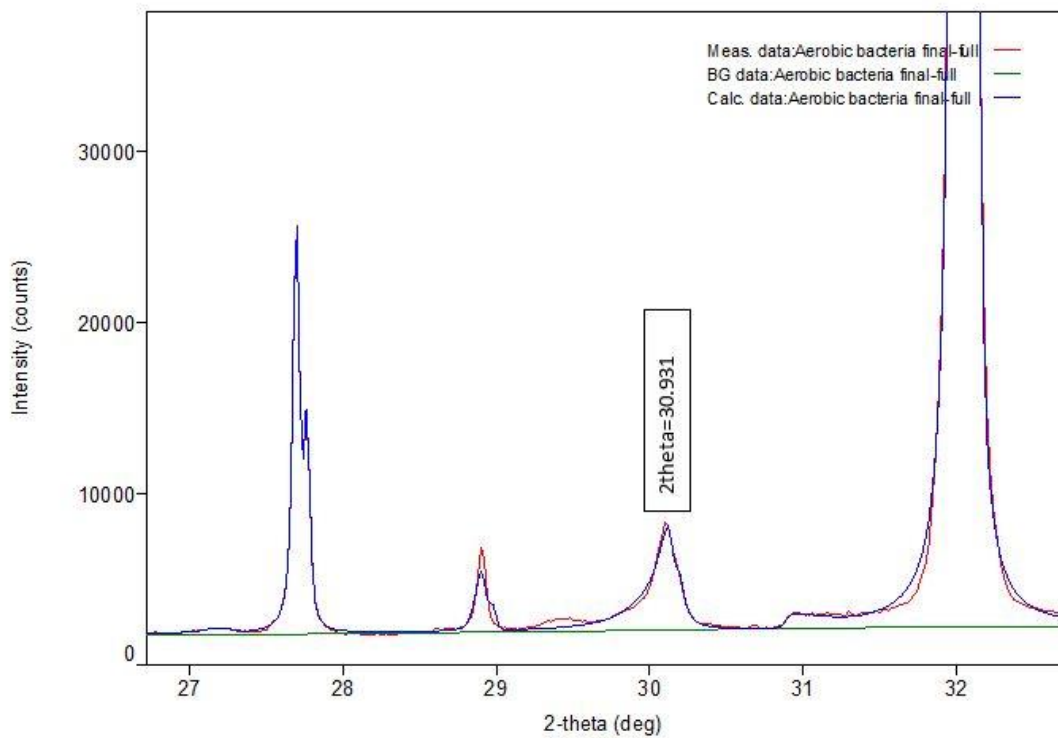


Figure 27. ATCC® Medium 2101: *Bacillus marismortui*, *Virgibacillus marismortui* (ATCC® 700626™) and amorphous carbonate. In this figure, XRD scan shows very weak signal for dolomite with the ($2\theta = 30.931$) value magnified from figure 2.

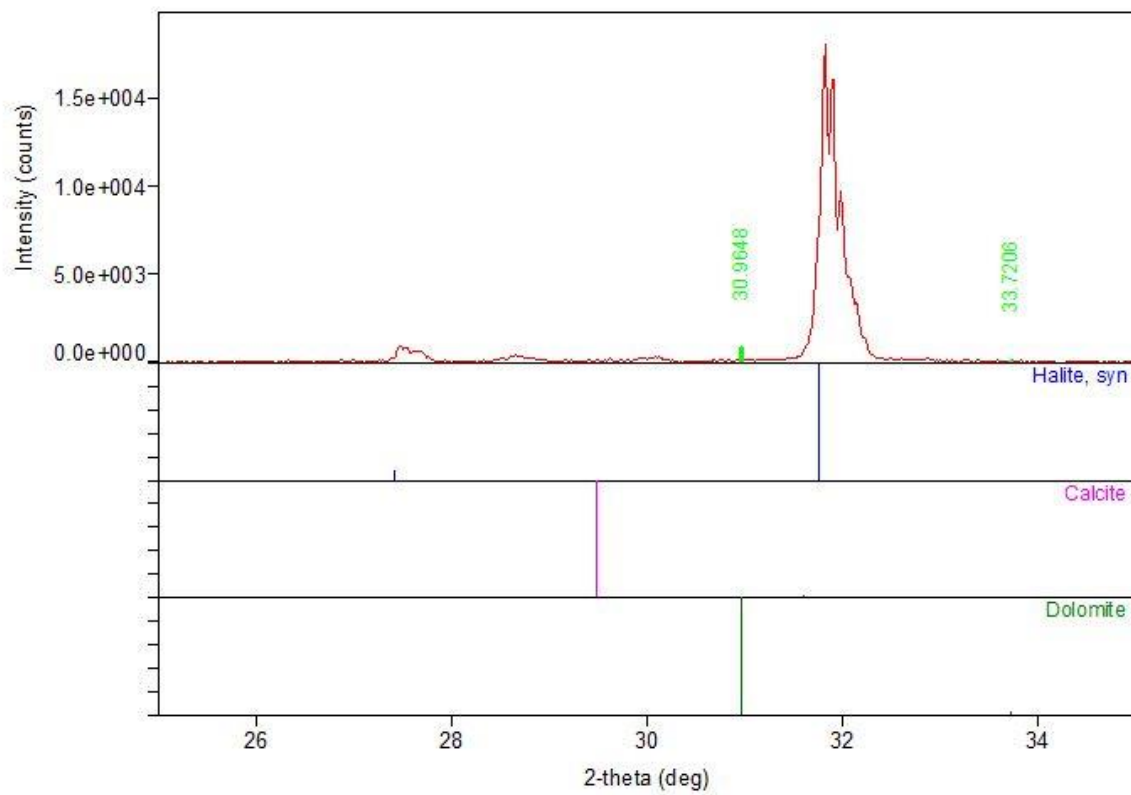


Figure18. ATCC® Medium 2101: *Bacillus marismortui* with amorphous carbonate only. In the above figure, preliminary XRD scan shows halite peak and potential carbonate peaks.

Escherichia coli

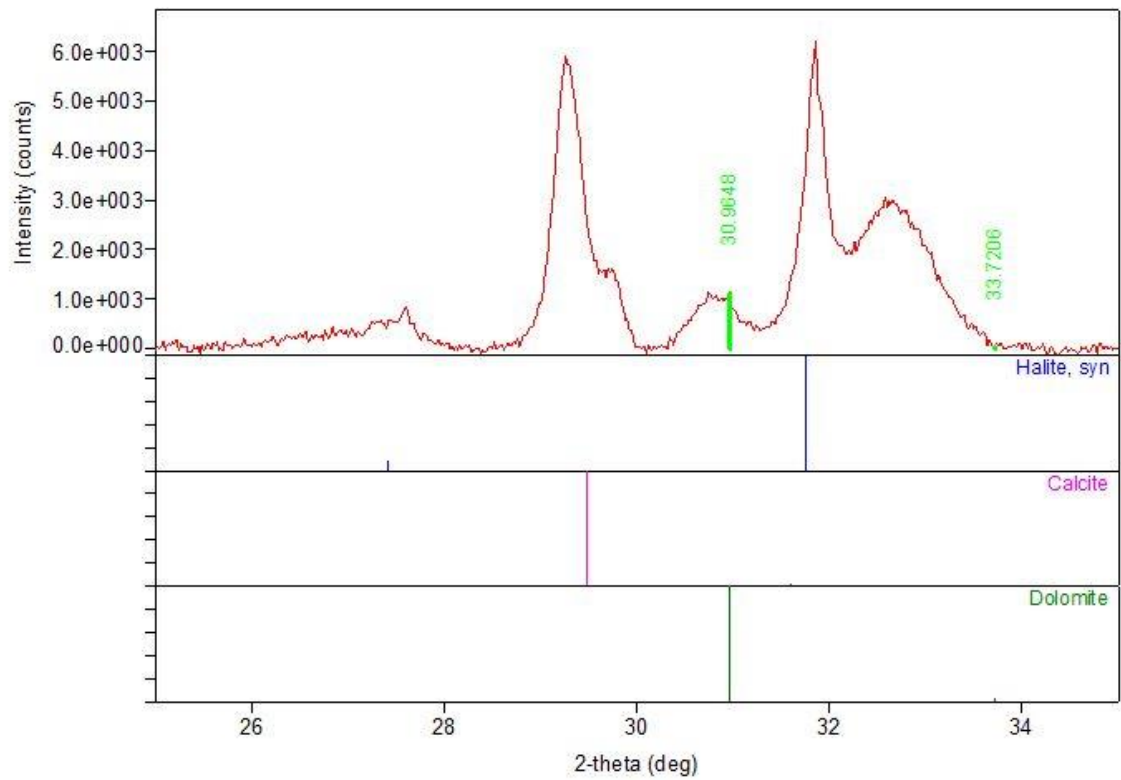


Figure 19. Luria-Bertani media with *Escherichia coli*, and amorphous carbonate. In the above figure, a preliminary scan was conducted that shows halite peak and calcite peak. Additionally, there appears to be a potential dolomite peak.

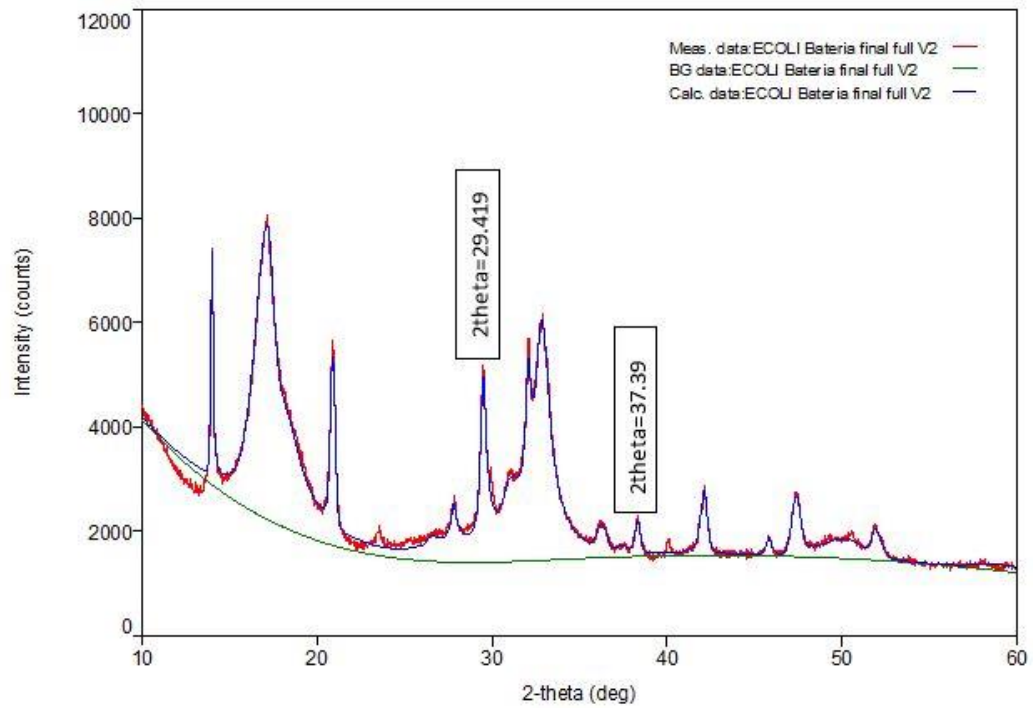
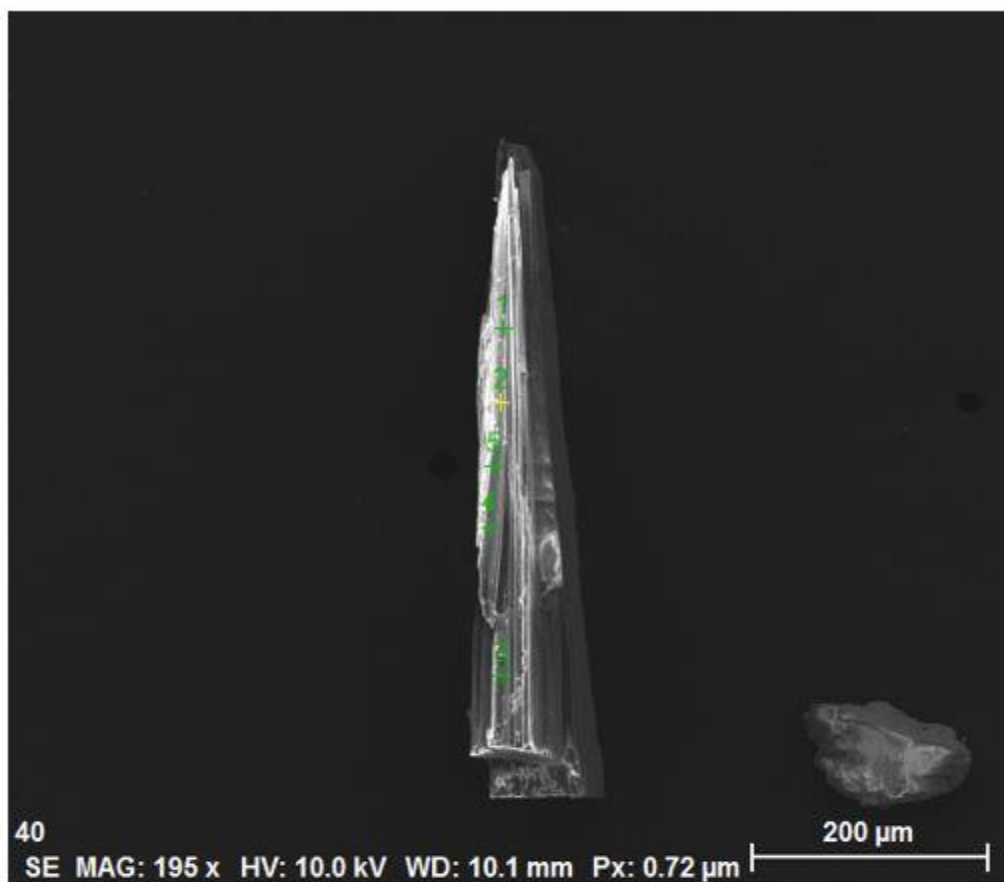


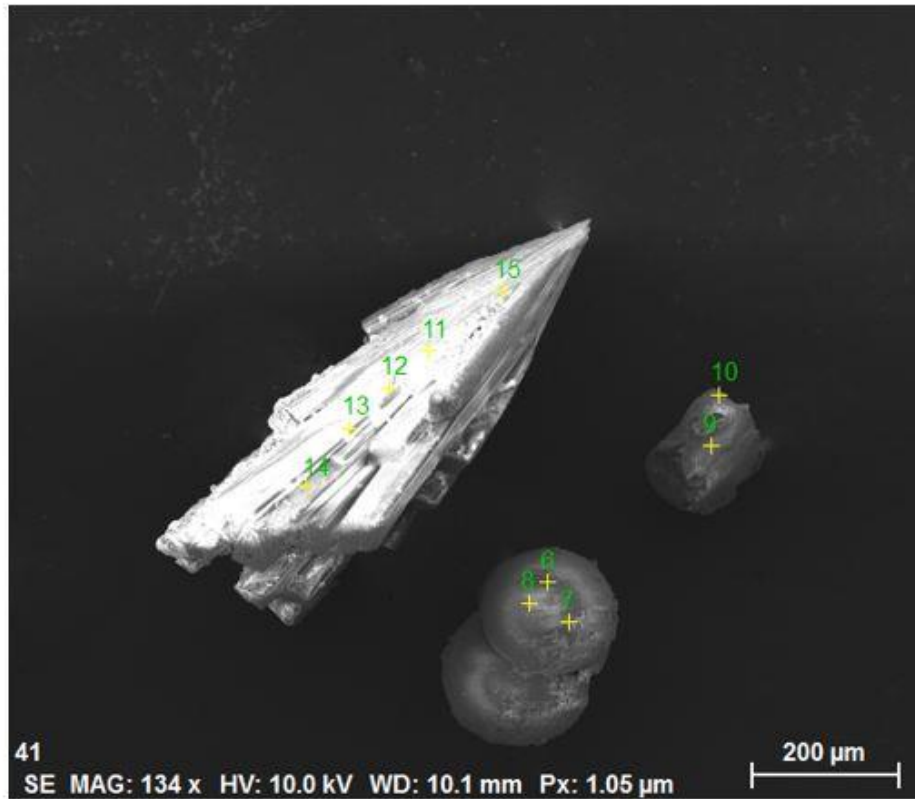
Figure 30. Escherichia coli, Luria-Bertani media and amorphous carbonate. Signal for precipitation of faint peak for dolomite ($2\theta=37.39$) and calcite ($2\theta=29.419$) are shown above from magnification of figure 4.



Norm. mass percent (%)

Spectrum	C	O	Mg
1	19.74	66.99	13.28
2	20.94	66.01	13.05
3	18.11	67.13	14.76
4	19.48	67.40	13.11
5	21.52	65.98	12.51
Mean value:	19.96	66.70	13.34
Sigma:	1.33	0.66	0.85
Sigma mean:	0.59	0.30	0.38

Figure 21. Luria-Bertani media with *Escherichia coli*, and amorphous carbonate. The SEM analysis shows crystal growth, in this figure showing magnesium carbonate



Norm. mass percent (%)

Spectrum	C	O	Na	Mg	P	Cl	Ca
6	11.24	57.32	0.85	2.61	0.86	0.59	26.54
7	8.72	38.69	2.61	5.94	1.72	1.10	41.22
8	9.56	44.89	1.08	2.92	1.33	1.43	38.78
9	19.17	48.34	-	2.07	-	-	30.43
10	15.71	62.25	-	2.16	-	-	19.87
11	41.41	48.92	-	9.67	-	-	-
12	47.99	48.05	-	3.96	-	-	-
13	21.09	63.46	-	15.45	-	-	-
14	13.88	68.77	-	17.35	-	-	-
15	30.57	58.03	-	11.39	-	-	-
Mean value:	21.93	53.87	1.51	7.35	1.30	1.04	31.37
Sigma:	13.69	9.51	0.96	5.75	0.43	0.42	8.78
Sigma mean:	4.33	3.01	0.30	1.82	0.14	0.13	2.78

Figure 22. Luria-Bertani media with *Escherichia coli*, and amorphous carbonate. In the figure, magnesium carbonate growth can be seen as well as the morphology of the bacteria.

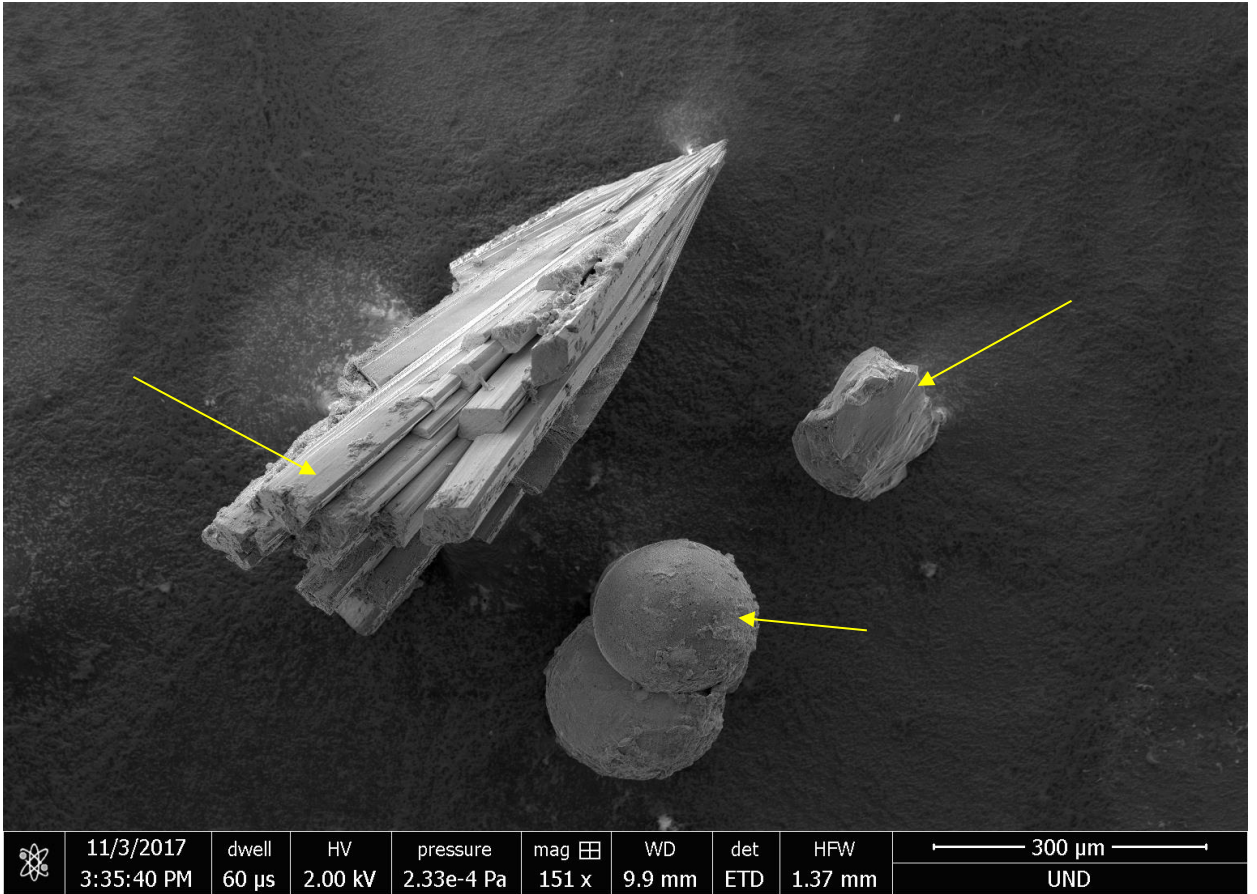


Figure 23. Luria-Bertani media with *Escherichia coli*, and amorphous carbonate. The above figure shows growth of carbonate crystals observed under SEM.

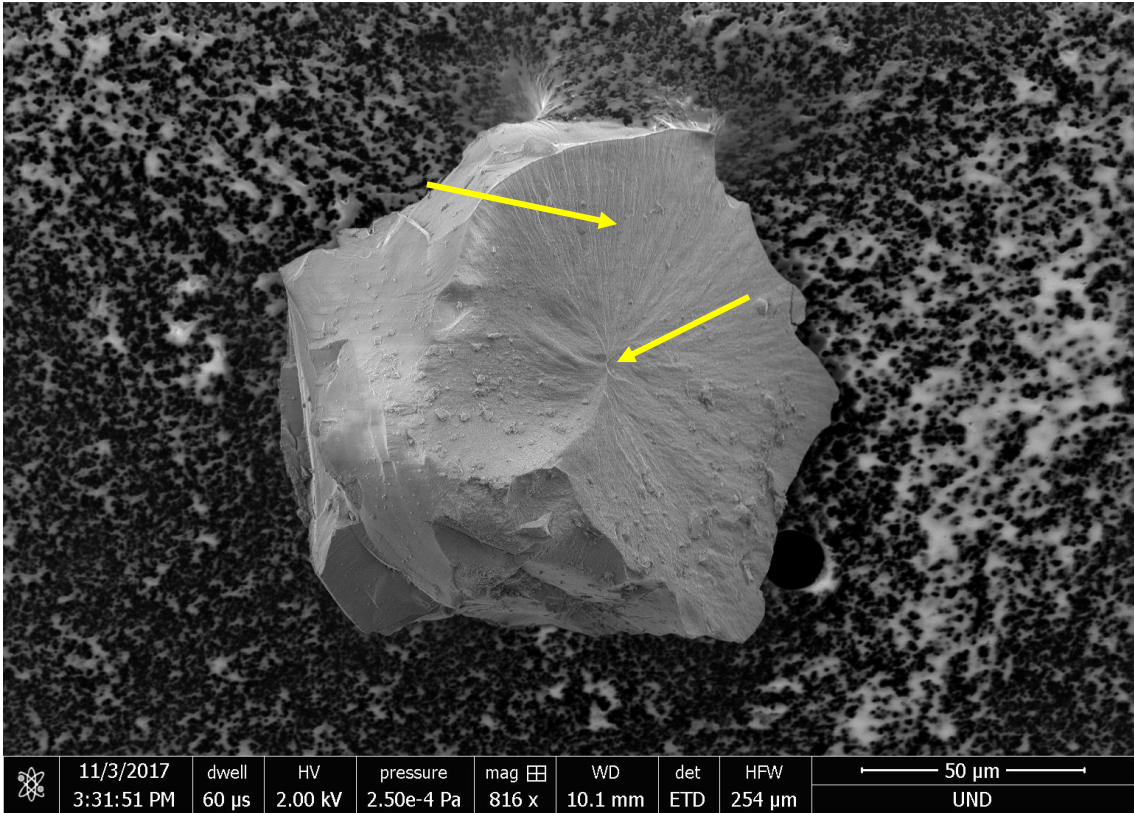


Figure 24. Luria-Bertani media with *Escherichia coli*, and amorphous carbonate. The above figure shows calcite with radial growth.

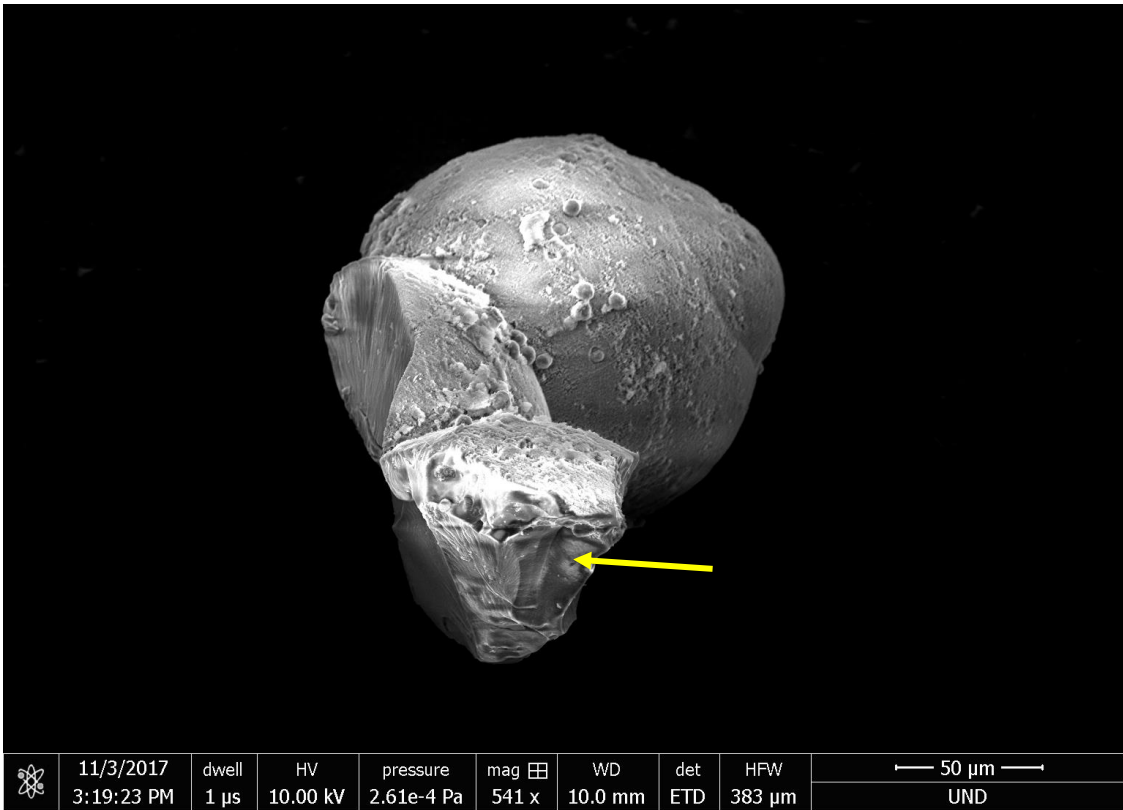


Figure 25. Luria-Bertani media with *Escherichia coli*, and amorphous carbonate. The above figure shows the growth and structure of carbonate budding off the bacterial surface.

Desulfovibrio desulfuricans subsp. *desulfuricans* (ATCC®29577™)

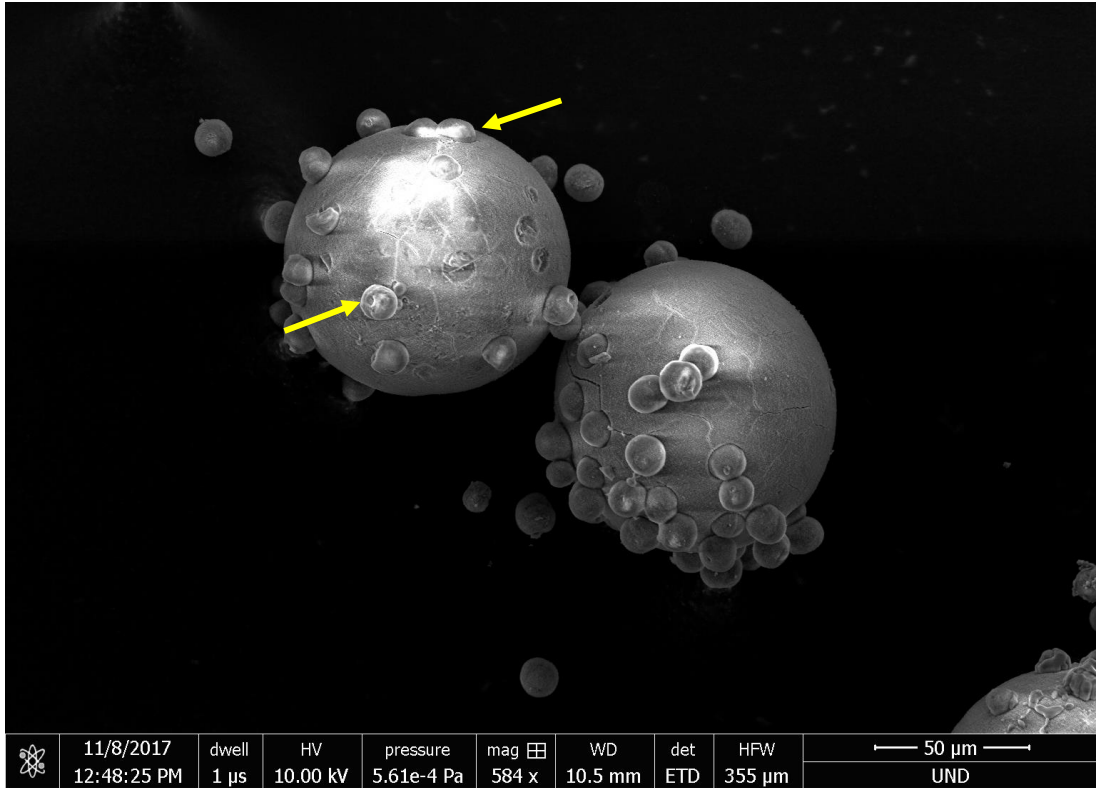


Figure 26- ATCC® Medium 1249: Modified Baar's media with *Desulfovibrio desulfuricans* subsp. *desulfuricans* (ATCC®29577™) and amorphous carbonate. In the above figure, carbonate can be seen growing and budding off bacterial surface.

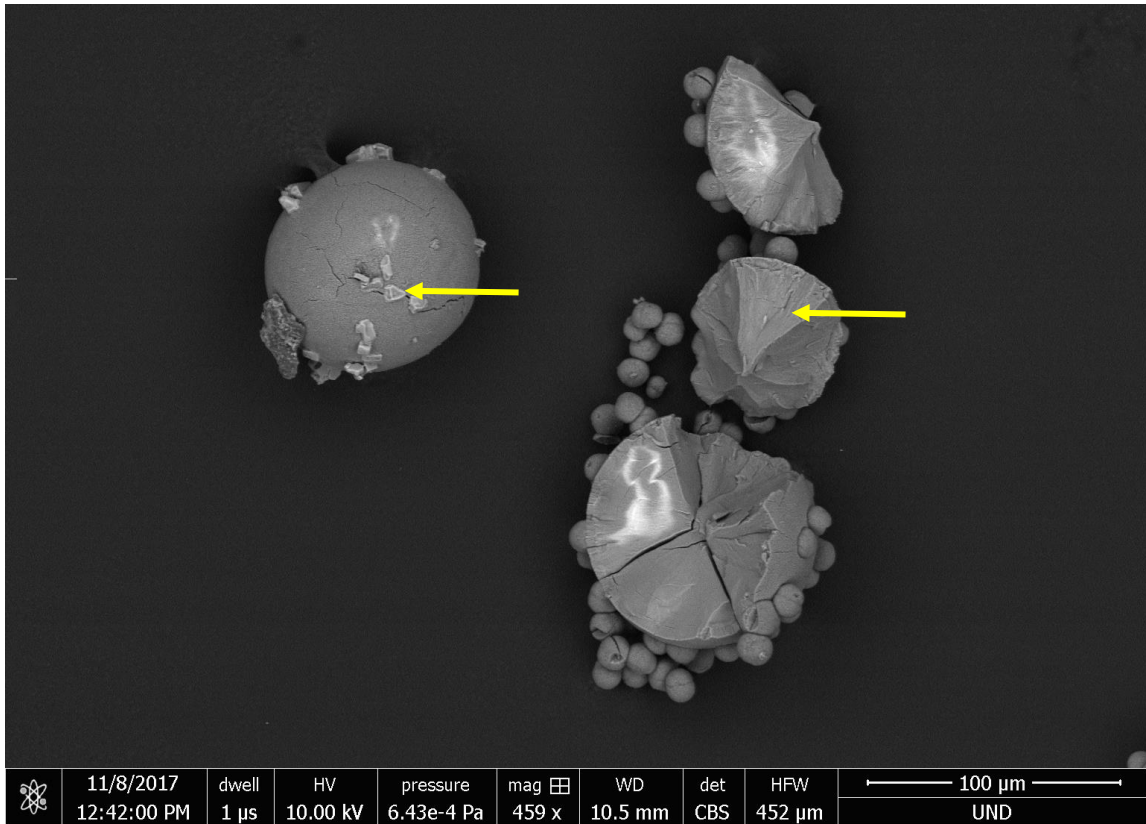


Figure 27. ATCC® Medium 1249: Modified Baar's media with *Desulfovibrio desulfuricans* subsp. *desulfuricans* (ATCC®29577™) and amorphous carbonate. In the above figure, carbonate can be seen budding off bacteria as well as calcite precipitate to the right.

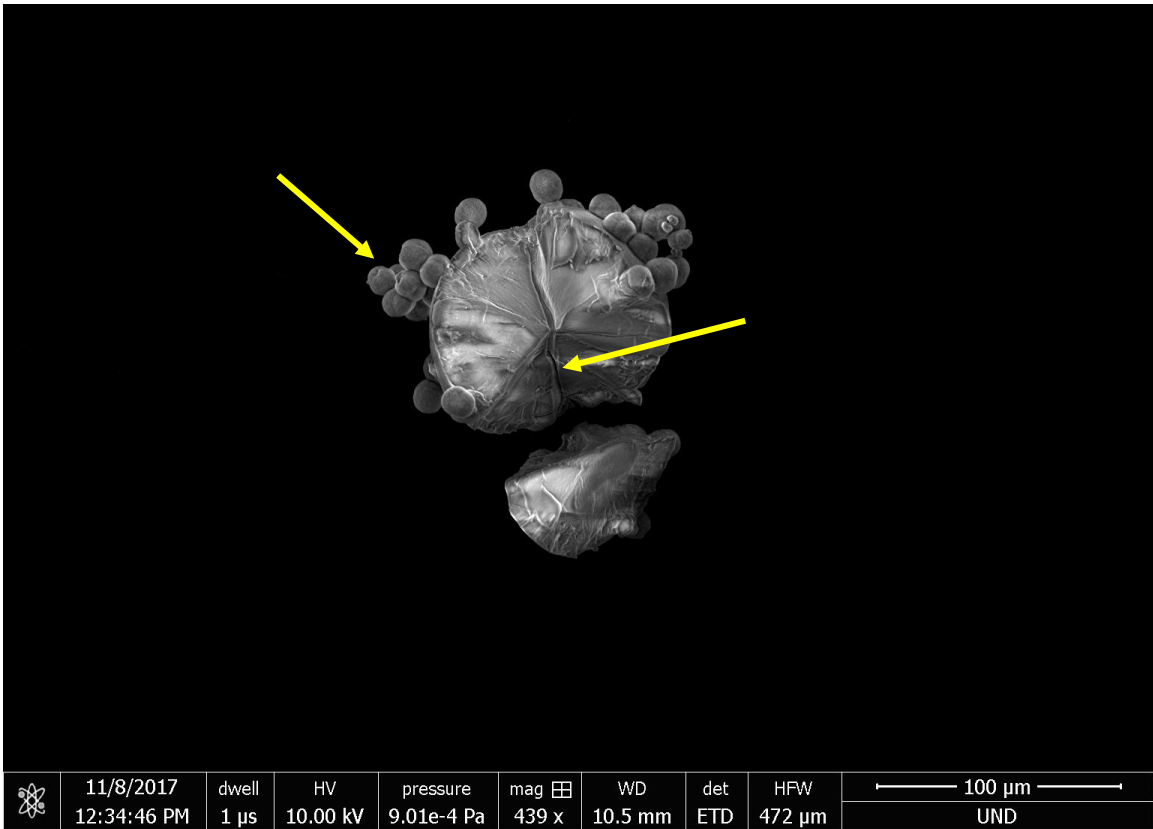


Figure 28. ATCC® Medium 1249: Modified Baar's media, *Desulfovibrio desulfuricans* *subsp. desulfuricans* (ATCC®29577™) and amorphous carbonate. In the above figure, calcite can be seen in the small clusters as well as the bigger piece of calcite in the center as indicated by the arrows.

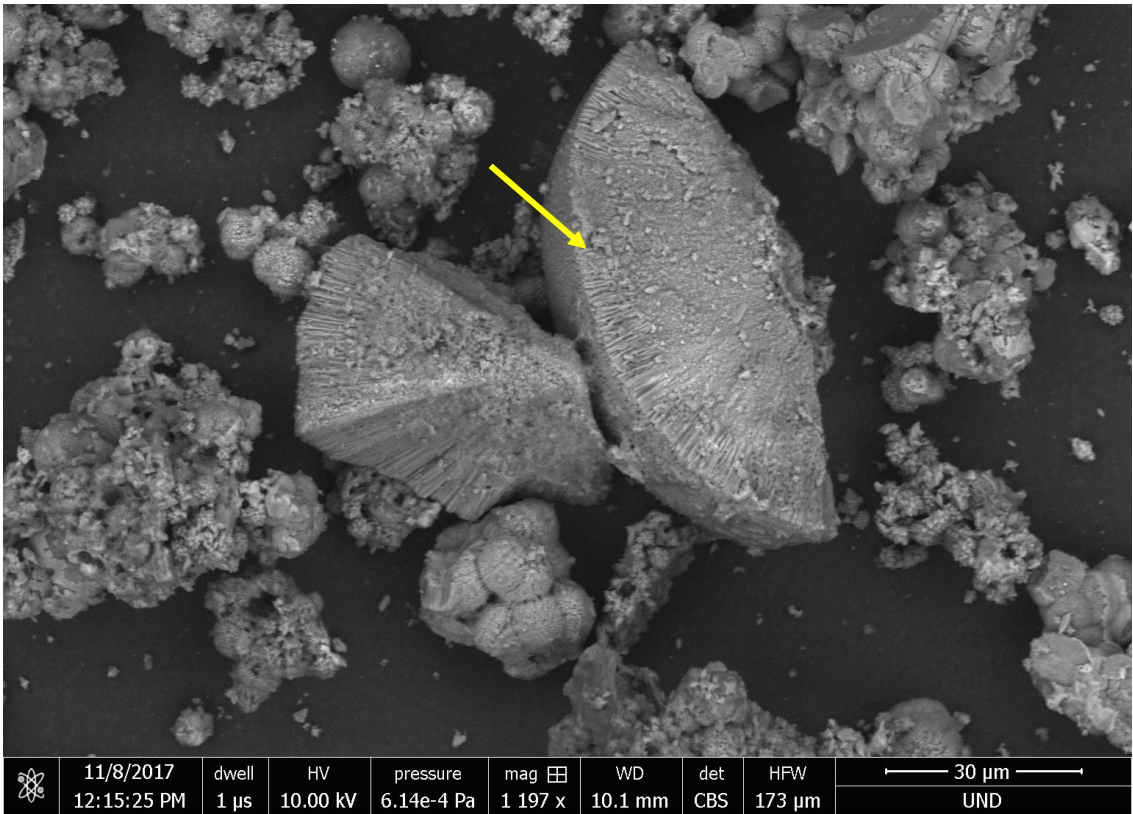


Figure 29. ATCC® Medium 1249: Modified Baar's media and amorphous carbonate only. In the figure, calcite can be seen in the center with radial growth patterns along the edges as indicated by the arrow.

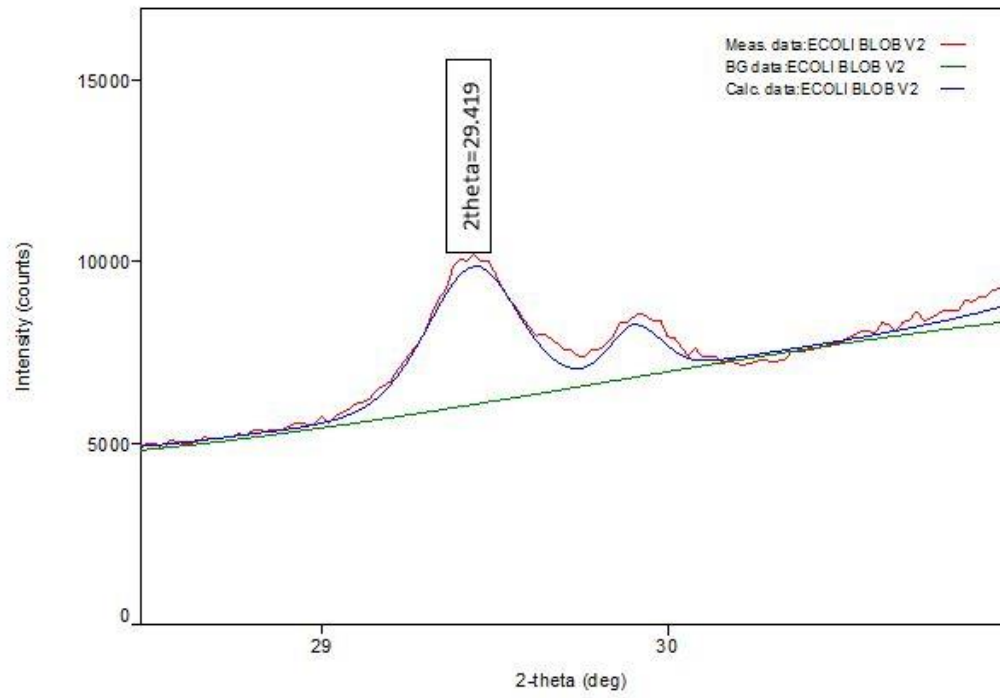


Figure 40. Luria-Bertani media and amorphous carbonate. In the above figure, there is a peak for calcite ($2\theta=29.419$), magnified from figure 5.

APPENDIX C

MINERAL DATA TABLE

Table 1. Data table showing standard XRD 2 θ peaks vs peaks from samples for the mineral dolomite with intensity and H, K, L. (1) and (2) are peaks for *Desulfovibrio desulfuricans subsp. desulfuricans* (ATCC®29577™), (6) for *Escherichia coli* and (4) for *Virgibacillus marismortui* (ATCC® 700626™).

Dolomite	Standard 2 θ	Sample 2 θ	Yes/No	H	K	L	Intensity
	30.86	30.78(1),30.57(2)	Yes	1	0	4	100
	37.35	37.27, 37.39(6)	Yes	1	1	0	11
	50.93	50.91(4)	Yes	1	1	6	12.85

Table 2. Data table showing standard XRD 2 θ peaks vs peaks from samples for the mineral calcite with intensity and H, K, L. (1) is peak for *Desulfovibrio desulfuricans subsp. desulfuricans* (ATCC®29577™), (5) and (6) are for *Escherichia coli*.

Calcite	Standard 2 θ	Sample 2 θ	Yes/No	H	K	L	Intensity
	29.42	29.41(1,5,6)	Yes	1	0	4	100
	31.46		No	0	0	6	2.43
	36	36.027(1)	Yes	1	1	0	13.71

Table 3. Data table showing standard XRD 2 θ peaks vs peaks from samples for the mineral magnesite with intensity and H, K, L. (5) and (6) are peaks for *Escherichia coli*, (2) is for *Desulfovibrio desulfuricans subsp. desulfuricans* (ATCC®29577™) and (3) is for *Virgibacillus*

Magnesite	Standard 2 θ	Sample 2 θ	Yes/No	H	K	L	Intensity
	32.67	32.79(5), 32.87(3)	Yes	1	0	4	100
	38.88	38.24(1)	Maybe	1	1	0	6.3
	51.66	51.6(6), 51.76(4)	Yes	0	2	4	5.33

marismortui (ATCC® 700626™).

Table 4. Data table showing standard XRD 2 θ peaks vs peaks from samples for the vaterite with intensity and H, K, L. No peaks were identifiable with the standards chosen.

Vaterite	Standard 2 θ	Yes/No	H	K	L	Intensity
	21.07	No	0	0	6	9.85
	24.42	No	1	1	0	56.83
	31.68	No	2	0	4	11.03
	40.37	No	2	0	8	9.38

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