Bio-Cleaning by *Desulfovibrio vulgaris* bacteria for black gypsum crust of archaeological stone.

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Abstract:

One of the most deteriorated layers which always appear on stone surface is the black layer, especially the one located in open area. Several methods were carried on the monuments to decrease the effect of the black crust phenomena, but none of these methods save the cultural heritage and the patina noble of the stone and the pigments in decorated stone. Through this survey, a self-cleaning conservation by (SRB) "sulfate reducing bacteria", particularly, *Desulfovibrio vulgaris*. The white layer calcium carbonate can be formed due to the transformation of the black calcium sulfate by *D. vulgaris* within 24h. The self-cleaning technology is safe for both the conservators and the archaeological sites, risk-free, accomplishment simplicity, applicable.

Key words: patina noble, Self-cleaning, Remediation, Black crust, *Desulfovibrio vulgaris*, Manial Palace, Sulfate reducing bacteria.

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1. Introduction:

The deteriorated black gypsum crust appeared on the cultural heritage stone surface when the polluted atmosphere reacted with the lime stone layer (Esbert 2001; Rodriguez-Navarro 2003).

Limestone consists of calcium carbonate as a main mineral, the insoluble calcium carbonate mineral converted into the dihydrate calcium sulfate ($CaSO_4.2H_2O$) as an interaction between a sulfuric acid (H_2SO_4) and the archaeological limestone (Ausset 2000; Bugini 2000; Tidblad 2012).

During the crystallization of gypsum, airborne pollutants precipitated in the mineral pores and blackening the surface. (De la Fuentea 2013).

In the last ten years, several applications carried over cultural heritage stone by using viable cells of bacteria which could remove the black sulfate layer from the carbonating stone (Cappitelli 2006; Bosch- Roig 2013a, b; Dhami 2014).

A special biological formulation containing bacteria such as *Desulfovibrio* sp. used for the self-cleaning methodologies carried on different type of stone and archaeological sites. (Cappitelli, 2007; Valentini 2010, Barbabietola 2012, Caneva 2017).

(Atlas 1988; Gauri and Chowdhury 1988), announced that *Desulfovibrio desulfuricans* could convert calcium sulfate into H_2S and the calcium Ca^{++} released after anaerobic reaction reacts with CO_2 to forms calcium carbonate $CaCO_3$, the new

formation of the calcium carbonate was a cleaning process and a consolidation process at the same time.

Avoiding sulfide precipitation, a kind of delivery system could be apply with the strain of bacteria, such as carbogel. (Webster and May 2006, Bosch- Roig 2015, Gioventù 2013).

Desulfovibrio vulgaris used by Gauri 1992, for the first time for cleaning marble statue deteriorated by gypsum, He immersed the statue in a growth medium containing bacteria for 84 hours. Consequently, he reported that this kind of treatment could only be used for small immersed objects.

In 1997, Ranalli, et al performed *D. desulfuricans* and *D. vulgaris* on two objects of marble and they used, as a delivery system, the inorganic material split and they applied the cleaning process for 36 hours.

In this study, *D. vulgaris* with a carbogel as a delivery system applied over the stone of Mohammed Ali palace for 24hours, The application performed over tissue paper as a poultice which could be a solution for using this methodology for large objects.

2. Materials and Methods

2.1 Bacteria, delivery system, and media

A closer look at this article, the *D. vulgaris* "ATCC 29579" used in the biocleaning process, this strain carried from MERCIN center in Ain Shams University.

The media of this strain was "DSMZ 63 medium" which consist of "($MgSO_4.7H_2O$ ≈2g/L; K_2HPO_4≈ 0.5 g/L; DL-sodium lactate≈ 2.0 g/L; Na_2SO_4≈1.0 g/L;

$CaCl_2.2H_2O$≈0.1g/L; NH_4Cl≈1.0 g/L; $FeSO4.7H_2O$≈ 0.5 g/L; yeast extract≈1.0 g/L; resazurin≈1.0 mg/L; ascorbic acid≈ 0.1 g/L; sodium thioglycolate≈ 0.1 g/L)" and incubated for 96h at 30°c under anaerobic conditions. (Ranalli 1997).

Carbogel® (CST, 801716, Vicenza, Italy) has been used as a delivery system to conserve perfect contact between the treated stone surface and the bacteria cells, and at simultaneously, it helped to remove extra bacteria cell after remediation.

Under the anaerobic condition in a glove box, the bacteria cell trapped in carbogel for 10min during the formation of the gel.

The application has been performed over tissue paper as a poultice and left for 24h, and then removed and cleaned by sterilized cotton swab.

2.2 X- ray diffraction Analysis (XRD)

XRD analysis determined the mineralogical composition of the precipitation calcium carbonate. The crystals examined by XRD powder diagrams ("Philips PW 1140 and Rigaku-Miniflex Ca 2005 diffractometers") coupled with a Ni Filter and a Cu-Kα radiation source. (Goldsmith 1961).

2.3 Stereomicroscope

"Wild Makroskop M420" stereomicroscope (Heerbrugg, Switzerland) attached with an Olympus OM1 camera (Chicago, USA) used for the observation of samples.

2.4 Scanning Electron microscope (SEM)

SEM micrographs obtained using ("Jeol JSM 5600LV Model Philips XL 30 attached to EDX Unit"), with accelerating power (voltage 30 K.V., and magnification 10x up to 400.000x).

2.5 FTIR Spectroscopy

FTIR analyses executed by a "Nicolet Nexus spectrophotometer (Washington, USA)", which combined with (Nicolet Continuum), "Graseby-Specac diamond cell" has been used to record the spectra.

3 Results

3.1 Stereomicroscope

The observation by stereomicroscope for the stone of Mohamed Ali Palace in El- Manial area, Cairo present that the stone is a dolomitic limestone, the grain appeared in curved faces and columnar grain as shown in Fig.1.

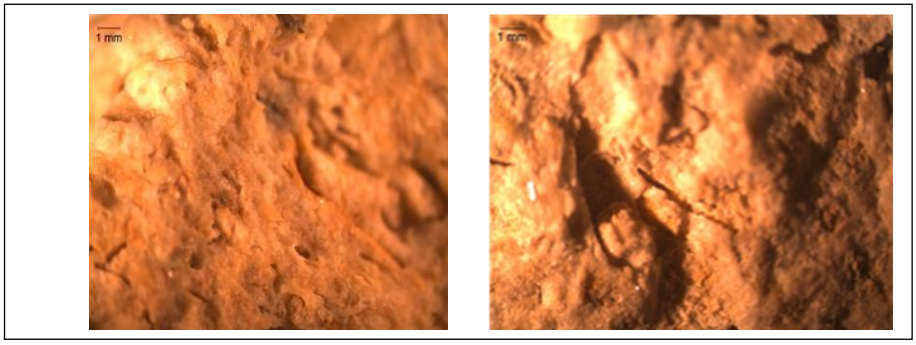

Fig.1 The sample grains of the dolomitic limestone under stereomicroscope

3.2 Fourier Transform Infrared Spectroscopy

FTIR spectra showed the presence of calcium sulfate di hydrate- Gypsum (3533, 3410, 1622, 1116 and 673 cm^{-1}). Calcium carbonate- calcite (1798, 1429, 876, and 712 c A . in addition to the Magnesium calcium carbonate- dolomite (2981, 2877, 2517, 1798, 876 cm^{-1}), as shown in (fig.2- a) but after the bio cleaning methodology (fig2-b) only dolomite and calcite presented with a traces of gypsum after the bio cleaning methodology.

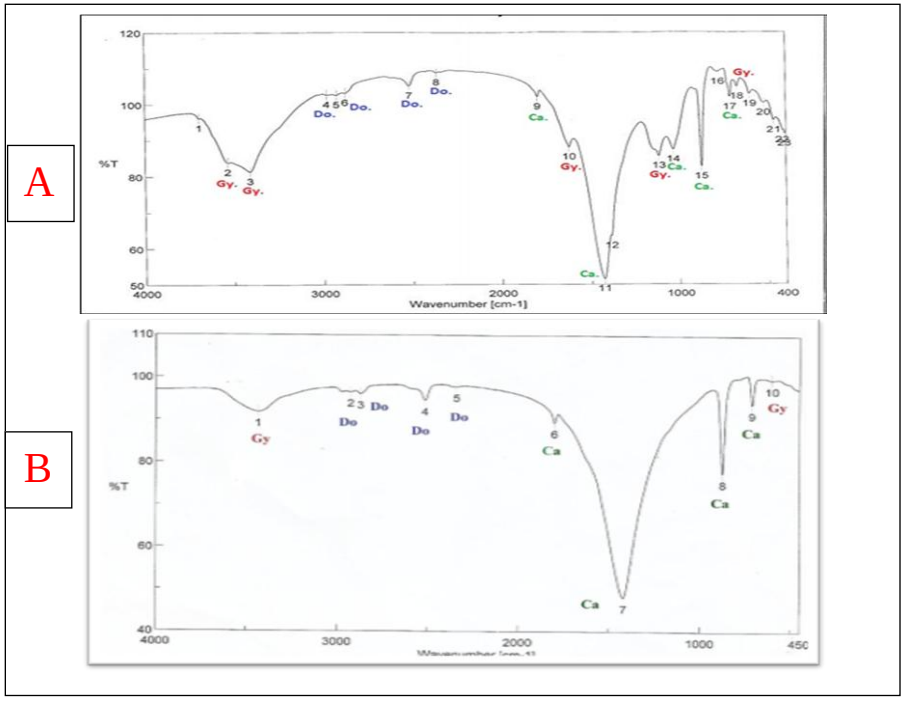

Fig.2(a) FTIR spectrum of the deteriorated black crust layer.
(b) FTIR spectrum of the sample after bio-cleaning.

3.3 X- ray diffraction Analysis (XRD)

The data analysis showed that the untreated stone consisted of 49% of Dolomite $CaMg(CO_3)_2$, 39%, Calcite $CaCO_3$, and 10%Gypsum $CaSO_4.2H_2O$, with a traces of Quartz SiO_2 1% as presented in Fig. (3-a).

After bio cleaning process, the data analysis presented only the minerals of the dolomitic limestone with a little amount of the deteriorated gypsum layer as shown in Fig (3-b).

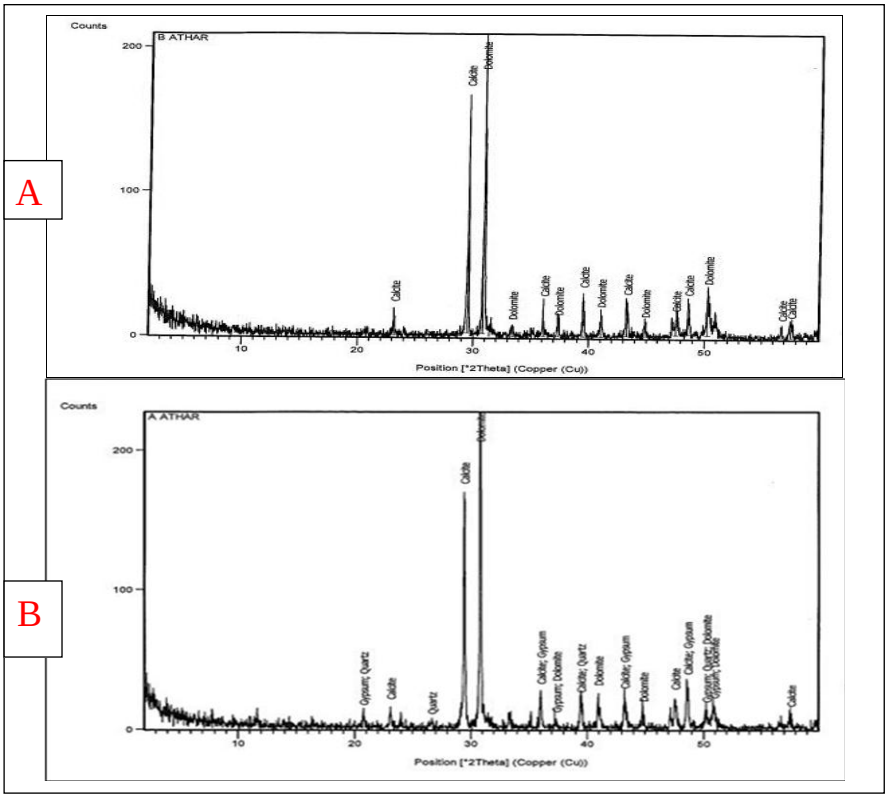

Fig.3 (a) The spectrum of untreated sample
(b) The spectrum of the treated sample after bio-cleaning process.

3.4 Scanning Electron microscope (SEM)

The SEM-EDX showed that the deteriorated stone sample consisted of calcite, Dolomite, and gypsum, as shown in Fig.4-a , while Fig 4-b showed that the calcium aion increase than before as white consolidated layer instead of the Gypsum layer.

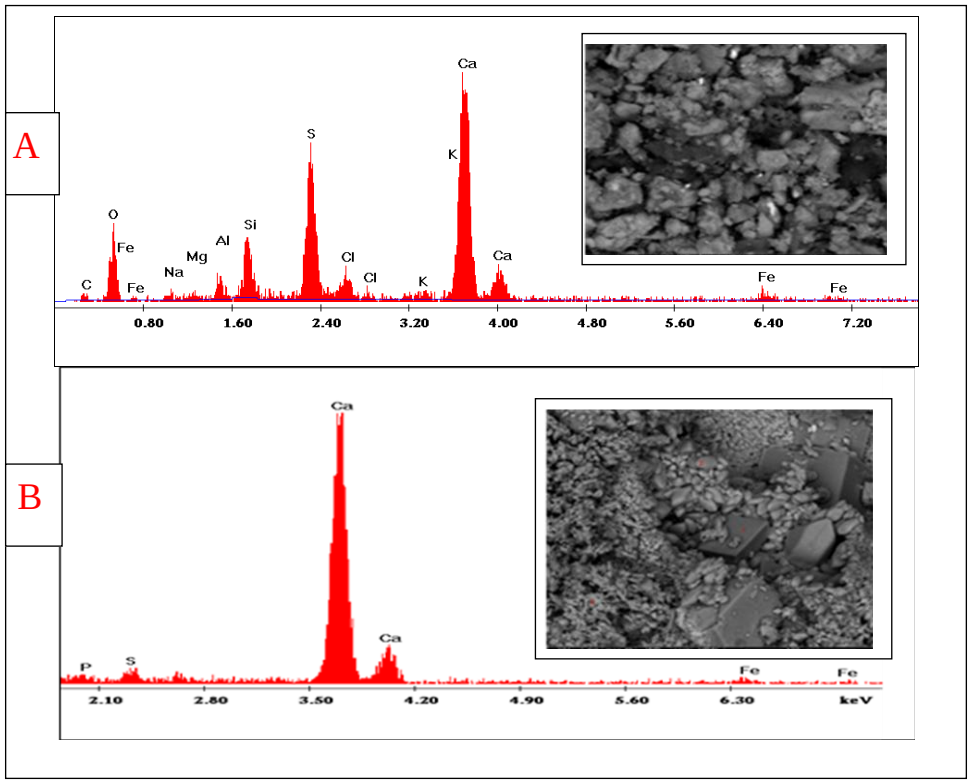

Fig.4 (a) SEM-EDX spectrum of the deteriorated black crust sample,
(b) spectrum of the black crust sample after bio-cleaning process,

3.5 Bio cleaning treatment

The bio-cleaning treatment process applied on the sample that previously taken from the same place, for the preparatory step. The untreated deteriorated sample, the first step of bio-cleaning process and the final step for the bio-cleaning process documentated as presented in fig.5 (a, b, c).

The final removal of the deteriorated black layer observed after two applications (12h for each one).

The FTIR, SEM- EDX, and XRD analysis implemented on the archaeological samples after the bio-cleaning treatment confirmed that the gypsum was almost completley removed, as shown before in fig.3 and fig.5.

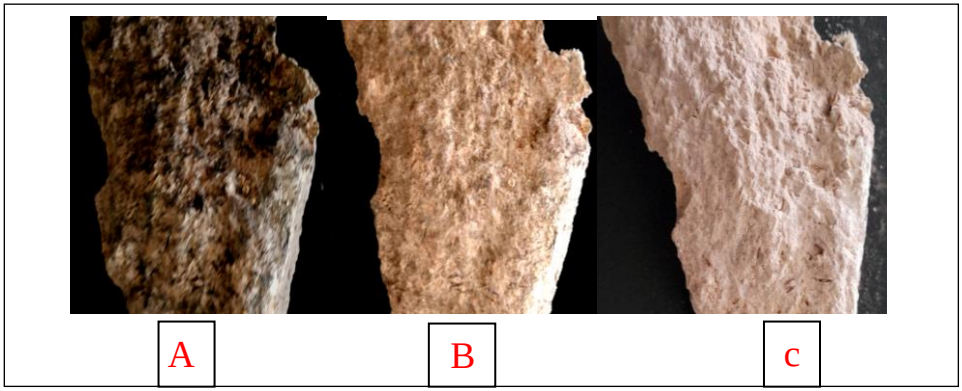

Fig.5 Bio-cleaning process of the archaeological sample (a) the deteriorated sample (b) after 12h of bio-cleaning (c) after 24h of bio-cleaning.

3.6 Application on Manial palace stone

To allow easy application of *D. vulgaris* sub.sp after the successful result that has been showed before, the *D. vulgaris*

sub.sp applied on Manial Palace stone, It has been kept in Carbogel® and applied over the tissue paper then covered by sterilized cotton swab and poly ethylene sheet, (preparing the bacterial cell with the delivery system made in an organic chemistry lab in Helwan University).

The Gel poultice left over the archaeological stone surface for only one day (24hour), and then the poultice has been removed. The remaining amount of the black gypsum removed by using a sterilized cotton swab damping with distilled water. The final result was a gorgeous noble patina, as seen in fig.6.

Fig. 6 The bio-cleaning process of the Manial Palace stone (a) deteriorated stone (b) treated stone after 24h of biological cleaning.

4 Discussion

Conceivably, the data analysis presented that the deteriorated black crust of the stone of the Manial Palace consisted of Gypsum layer.

The examination by steriomicroscope demonstrated that the stone of Mohamed Ali palace is a dolomitic limestone with a curved grain and columnar grain. The XRD presents that calcium sulphate di hydrate was one of the major component in the deteriorated black layer. In the other hand, the SEM-EDX showed that the sulfur element spectrum in the deteriorated layer was higher than the second analysis after bio-cleaning. The FTIR analysis confirmed that the presence of gypsum existed while after bio-cleaning process the FTIR spectrum showed that the gypsum component decreaced.

The bio-cleaning process by *D. vulgaris was* applied previously to remove sulfates element from marble (Cappitelli 2005, 2007). Also, it implemented on limestone that was more porous than the marble (Gioventu 2011, Polo 2010;), but non of their work did not applied on archaeological sites.

In the current study, *D. vulgaris* with a carbogel as a delivery system performed successfully on a deteriorated limestone sample taken from Manial Palace by using poultice technique for two times each one for twelve hours.

The XRD data for the stone sample after bio-cleaning showed that the gypsum peak decreased to the only trace, the EDX approved that the sulfur spectrum dropped and the calcium range increase.

The examination by SEM revealed a new formation of calcium carbonate with reference to “Gauri and Chowdhury 1988” the presence of calcium carbonate after bio-cleaning by *D. vulgaris* (SRB) is due to the fact of microbial activity.

The released of calcium ions after breaking down of gypsum has been reacted with carbon dioxide that produced from the respiration of bacteria leads to a new calcium

carbonate formed. This step is a cleaning and consolidated step. (Alfano 2011, Baebabietola 2012)

According to the experimental results, *D. vulgaris* with the carbogel has performed on the Manial Palace stone on a Japanese paper and covered with poly ethylene sheet then left for 24h.

After bio-cleaning process, a Nano silver particles with a size of 10nm used for sterilization of the archaeological stone to make certain that there was no more vaiable bacteria cell alive.

5 Conclusion

Carbon dioxide is one of the most polluted atmosphere gases that effect on the calcareous materials which result in presents of the black crust phenomenon. The Mohamed Ali palace stone was deteriorated by the black crust phenomenon which is difficult to remove. This work showed a new biotechnological process by *D. vulgaris* which would provide a valuable solution for this problem. The bio-cleaning treatment by D. vulgaris remove the black crust from limestone with no risk and consolidated the surface by a new formation of the calcite.

This technology has many advantages its safe, adhesion capabilities, efficiency, naturalness, and easy to use.

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