SUITABILITY OF BIOCEMENT PRODUCED BY ISOLATED MICRO-ORGANISMS AS A CEMENTING MATERIAL IN MORTAR

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ABSTRACT: This research aimed at partial replacement of Ordinary Portland Cement (OPC) with biocement obtained from biocementation processes induced by microorganisms for production of mortar. Urease positive bacteria were identified, cultured and feed into solutions containing a sterile mix of $CaCl_2$ (0.75M) and urea (1M); 50 litres of this was produced. Mortar prisms of 40 mm x 40 mm x 160 mm were cast using a mix ratio of cement to sand of 1:3. A total of 54 prisms were cast and cured in water for 7, 21 and 28 days. OPC was replaced by biocement containing 20% pulverized fired clay at 10, 20, 30 and 50%. Bacillus Licheniformis isolated from bovine faeces was identified as urease positive micro-organism suitable for calcite precipitation producing at a rate of approximately 0.67g/hour. Results revealed that bacteria cells increased the compressive strength of mortar by 20% and the combined effect of bacteria cells, pulverized fired clay and biocement increased the compressive strength by 43.6% at 10% replacement. It was generally concluded that bacillus Licheniformis can precipitate $CaCO_3$ through urea hydrolysis and bacteria cells as well as biocement containing 20% pulverized fired clay at 10 and 20% partial replacement increased the compressive strength of mortar, therefore suitable for mortar production where design compressive strength are desired.

Keywords: Biocement, Calcium Carbonate, Micro-Organisms, Bacteria, Mortar

I. INTRODUCTION

Sandcrete mortar is generally used for load and non-loading masonry block production in most parts of the world. For a long time in Nigeria, sandcrete blocks are manufactured in many parts of the country without any reference to suit local building requirements or good quality work (Oyekan and Kamiyo, 2008). Sandcrete blocks are the commonest and most used masonry walling units in Nigeria. The most essential and expensive constituent of the block is cement needed to give acceptable quality fire resistance, sound insulation, compressive and shear strength etc. required by various standards. (Okafor and Ewa, 2012).

Portland cement-based materials are the most widely used construction materials. They are complex composite materials, which are strong in compression but weak in tension. Cement refers to binding agents that are mixed with water to produce a cementing paste. Biocement therefore refers to $CaCO_3$ deposit that is formed due to microorganism activity in the systems which are rich in calcium ions (Sri, Reddy, Manjusha & kumar, 2015).

Micro-organism and microbial mediated mineralization processes are active in almost every environment on earth (Lopez-Garcia, Kazmierczak, Benzerara, Kempe and Guyot, 2005; Shen, Buick and Canfield, 2001). In natural environments, chemical $CaCO_3$ precipitation is accompanied by biological processes, both of which often occur simultaneously or sequentially. Microbes from soils and aqueous media have been reported to induce the precipitation of calcium carbonate mineral phases in both natural and laboratory settings (Lian, Hu, Chen, Ji and Teng, 2006). Hence, microbial activity is regarded as an important player in the formation of carbonate sediments and soil carbonate deposits (Rivadeneyra, Delgado, Ramos-Cormenzana and Delgado, 1998). A number of studies have investigated carbonate mineralization induced by microbes (Wright and Oren, 2005) including that by soil bacteria (Cacchio, Ercole, Cappuccio and Lepidi, 2003; Stocks-Fischer, Galinat and Bang, 1999). Microorganism-induced sand bioconsolidation has also been previously considered for sand improvement (Whiffin, van Paassen and Harkes, 2007; DeJong, Mortensen, Martinez and Nelson, 2010).

II. LITERATURE REVIEW

Different bacterial species have previously been detected and assumed to be associated with natural carbonate precipitates from diverse environments. The primary role of bacteria in the precipitation process has subsequently been ascribed to their ability to create an alkaline environment (high pH) through various physiological activities (Douglas and Beveridge, 1998; Ehrlich, 1998; Castanier, Le Metayer-Levrel and Perthuisot, 1999; Castanier, Le Metayer-Levrel and Perthuisot, 2000; Fujita,

Ferris, Lawson, Colwell, and Smith, 2000). Many organisms use urea as a source of nitrogen by importing urea into the cell's cytoplasm. One of the most robust ureolytic bacteria is *Sporosarcina pasteurii* (formerly known as *Bacillus pasteurii). S. pasteurii* is an aerobic, spore forming, rod shaped bacterium. It uses urea as an energy source and produces ammonia which increases the pH in the environment and causes Ca^{2+} and CO_3^{2-} to be precipitated as $CaCO_3$ (Kroll, 1990; Stocks-Fischer et al., 1999; Chahal, Siddique and Rajor, 2012).

Recent studies reveal that concrete fed with calcite precipitating bacteria cells have the ability to continually produce calcite to seal up cracks. Autogenous crack-healing capacity of concrete has been recognized in several recent studies (Neville, 2002; Reinhardt and Jooss 2003; Li and Yang, 2007; Edvardsen, 1999). Micro cracks with widths typically in the range of 0.05 to 0.1 mm have been observed to become completely sealed particularly under repetitive dry/wet cycles. The mechanism of this autogenous healing is mainly due to secondary hydration of partially or non-reacted cementing particles present in the concrete matrix. Due to capillary forces, water is repeatedly drawn into micro cracks under changing wetting and drying cycles, resulting in expansion of hydrated cement particles due to the formation of calcium silicate hydrates and calcium hydroxide (portlandite). These reaction products are able to completely seal cracks provided the crack width is small. Larger sized cracks can only be partially filled due to the limited amount of unhydrated cement particles present, thus resulting in only a thin layer of hydration products on the crack surface. With respect to crack-sealing capacity, a process homologous to secondary hydration of cement particles is the process of carbonation. This reaction is also expansive as atmospheric carbon dioxide (CO_2) penetrates and reacts with calcium hydroxide particles present in the concrete matrix to produce various calcium carbonate minerals such as calcite, aragonite and vaterite. From the perspective of durability, rapid sealing of particularly freshly formed surface cracks is important as this hampers the ingress of water and other aggressive chemicals into the concrete matrix. Several chemicals such as sulphates, chlorides and acids are known to dramatically increase concrete matrix degradation and corrosion of embedded steel. The development of a self-healing mechanism in concrete that is thus based on a potentially cheaper and more sustainable material than cement which is both economical and environmentally beneficial for both economy and environment.

III. MATERIALS AND METHODS

The Ashaka brand of Ordinary Portland Cement (OPC) manufactured by Ashaka Cement Company Plc, was used for this research. Basically for mortar, only fine aggregate is required and was used for this research. The sand was obtained from natural quartzite river dredged sand which is free from deleterious materials in Bauchi state, Nigeria. Pottery Clay (mixed with kaolin) was sourced from the ceramic section of the Abubakar Tafawa Balewa University (ATBU), Bauchi and heated at 800 ^{0}C for 4 hours in a kiln, pulverized and sieved.

The water to be used for the production of the mortar was portable water and bacteria suspension of 0.1M $CaCl_2$.

Upon culturing of bacteria, the species that had the best urease activity were isolated and inoculated for precipitation of calcium carbonate. The calcium carbonate produced was obtained as a solution and mixed with pulverized clay. This mixture was thoroughly mixed further with the fine aggregate to form the biocement used in the production of mortar blocks wherein OPC is partially replaced.

3.1 Isolation of Urease Producing Bacteria

To enrich the samples for urease-producing bacteria, 1 g of each solid sample was inoculated into 50 ml of Brain heart infusion broth and incubated at 37^{0}C for 24 hours. For liquid samples, 0.5ml of the sample was collected into the enrichment media of 4.5 ml of nutrient broth.

To screen for pure isolates, the enrichment cultures were streaked on blood agar plates which is used for the differentiation of microorganisms on the basis of urease production. The plates were incubated at 37^{0}C for 48 hours. A loop full of colonies was placed into urea agar to observe for colour change.

3.2 *Experiments on Biocement*

All tested isolates were cultivated in a nutrient broth. Before the cultivation, medium was sterilized at 121 ^{0}C; the organisms were grown at 37 ^{0}C for 24 hours in an incubator. Biochemical tests were carried out in order to classify the bacteria. The various biochemical tests were: gram staining, motility, utilization of citrate, glucose, arabinose, mannitol, xylose, indole, gelatine hydrolysis and urease

The classification was done using advanced bacteria identification software (ABIS) online. The bacteria were stored while being suspended in their growth medium in a fridge at 4 ^{0}C prior to use (Van Paassen, 2009, Henriques, 2011).

Two components of liquid were used: (1) cultured liquid (bacterial suspension) with addition of $CaCl_2$ to a concentration of 0.1 M; (2) calcium chloride and urea solution contained 82.5 g/L (0.75 M) of $CaCl_2$ and 60.06 g/L (1M) of urea. Little amount of the second liquid was taken and tested for the presence of $CaCO_3$ by adding little drops of HCl acid to observe for effervescence of CO_2 gas.

The concentration of $CaCO_3$ in the biocement solution was determined using titration with Hydrochloric acid (HCl) 0.5M used and Eriochrome Black T as indicator.

3.3 Mortar

The mortar blocks were produced with 0, 10, 20, 30 and 50 per cent replacement by weight of biocement to Portland cement and specimens designated as B10, B20, B30, and B50 accordingly. Two types of control specimen of 100 per cent OPC were produced; one with ordinary water (A) and the other with bacterial suspension of 0.1M of $CaCl_2$ (Z). The specimens sizes are 40 mm x 40 mm x 160 mm prism according to BS EN 196-1 (2005). They are cured for 7, 14 and 28days.

IV. RESULTS AND DISCUSSION

The various samples collected showed microbial growth; however only the *Bacillus* was positive to urease therefore confirming previous research that most bacilli are urease positive. The biocement produced when tested for the presence of $CaCO_3$ showed effervescence of gas which ordinary will be CO_2 gas. Hence more biocement solution of 50 litres was produced.

	Gram Reaction	Motility	Citrate	Glucose	Arabinose	Mannitol	Xylose	Indole	Gelatine Hydrolysis	Urease	Closely Related to
Bc	NA	NA	NA	NA	NA	NA	NA	NA	NA	-	***Unknown***
Bw	NA	NA	NA	NA	NA	NA	NA	NA	NA	-	***Unknown***
Bs	NA	NA	NA	NA	NA	NA	NA	NA	NA	-	***Unknown***
Bv	$+^R$	+	+	+	+	+	+	-	+	+	***Bacillus Licheniformis ~ 94%***

Key: NA Test not applicable, $+^R$ Gram positive Rod, - Negative Test result, + Positive Test result

Table 1: Identification of Isolates

4.1 Concentration of Calcium Carbonate

The concentration of the $CaCO_3$ produced microbially was determined by titration with 0.5M HCl. The concentration was calculated from balanced equations which estimated a concentration of 32.03g/l of $CaCO_3$ present in the biocement liquid as against a theoretical concentration of 74.4g/l (i.e. for complete reaction).

$$CaCl_{2(s)} + CO(NH_2)_{2(s)} + 2H_2O_{(l)} \xrightarrow{\text{UREASE}} CaCO_{3(aq)} + 2NH_4Cl_{(aq)}$$

1

4.2 Hardened Mortar

The flexural strength for controls made with ordinary water and control made with bacterial suspension liquid decreased with increase in the age of curing up to 28 days. For 10% partial replacement the flexural strength increased from 5.68Nmm^{-2} at 7 days to 6.02 Nmm^{-2} at 21 days and then decreased to 5.85 Nmm^{-2}. For 20, 30 and 50% replacement the flexural strength increased steadily achieving maximum strength at 28 days of curing. The result of the flexural strength is presented in table 2 and the relationship between the biocement content and the corresponding flexural strength with curing days is illustrated in figure 1.

Unlike the flexural strength, the Control made with ordinary water attained 99.29% (42.2 Nmm^{-2}) of design grade strength of cement which is 42.5 Nmm^{-2} 28 days of curing. Control made with bacteria suspension liquid attained 119.53%. 10, 20, 30 and 50% partial replacements attained 142.59%, 112.94%, 82.82% and 74.12% respectively. The best result for compression was seen at 10% (B10) replacement of OPC with biocement with 24.6, 58.5 and 60.6 Nmm^{-2} at 7, 21 and 28days curing periods conforming to BS 12 (1996) which gives the range of compressive strength of 16Nmm^{-2} at 3 days to a maximum of 62.5Nmm^{-2}.

As investigated by Reddy, et al (2010), the compressive strength of cement mortar reached the maximum with the addition of *bacillus subtilis* bacteria for a cell concentration of 10^5 cells per ml of mixing water with an of increased up to 14.92% at 28 days. Chahal et al, (2012), obtained a maximum increase of 22% in compressive strength and four times reduction in water absorption was observed with a 10^5 cells/ml of bacteria concentration. For Achal et al (2011), bacterial cell enhanced compressive strength up to 19% compared to control specimens of mortar.

The increase in compressive strength is mainly due to consolidation of the pores within the microstructure of the mortar cubes. In the case of this research, the high improvement in compressive strength is probably due to sufficient deposition of $CaCO_3$ present in the bacterial suspension and within the pores of cement–sand matrix. The presence of the fired clay which is Increase was noticed at 21 and 28 days curing periods. The optimum replacement for compressive strength was at 10% (B10) with an increase of 42.6% and 19.5% increase of control with bacteria cells compared to design compressive strength. rich in aluminium, silicon and iron can also be a contributing factor to this high improvement.

There was no increase in compressive strength of mortar prisms with partial replacement within 7 days of curing compared to the controls due to slow strength development.

Table 3 under shows the result of the compressive strength and figure 2 illustrates the relationship between the biocement content versus the compressive strength reached at given curing period.

	Failure Load (kN)				**Flexural Strength (Nmm^{-2})**
	1	2	3	Average	
			7 days Cured		
A	2.5	2.7	2.6	2.60	6.71
Z	2.6	2.5	2.4	2.50	6.45
B10	2.1	2.3	2.2	2.20	5.68
B20	1.9	2.0	1.9	1.93	4.99
B30	1.9	1.9	1.8	1.87	4.82
B50	1.7	1.7	1.8	1.73	4.47
			21 days Cured		
A	1.7	2.3	2.4	2.13	5.50
Z	2.1	2.5	2.2	2.27	5.85
B10	2.2	2.5	2.3	2.33	6.02
B20	2.3	2.3	2.3	2.30	5.93
B30	2.3	2.2	2.3	2.27	5.85
B50	2.2	2.1	2.1	2.13	5.50
			28 days cured		
A	2.4	2.5	2.5	2.47	6.36
Z	2.2	2.4	2.5	2.37	6.11
B10	2.1	2.4	2.3	2.27	5.85
B20	2.3	2.3	2.4	2.33	6.02
B30	2.4	2.1	2.3	2.27	5.85
B50	2.2	2.1	2.4	2.23	5.76

Flexural Constant = $(1.5 \times 110 \times 10^3)/ 40^3 = 2.58mm^{-2}$

Table 2: Flexural Strengths of Mortar Prisms

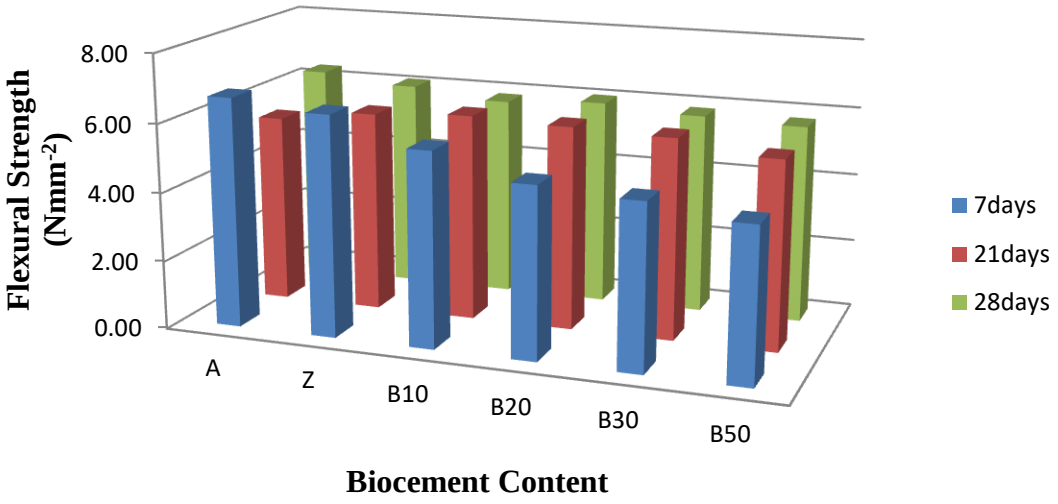

Figure 1: Bar Chart Showing the Flexural Strength at given Curing Period

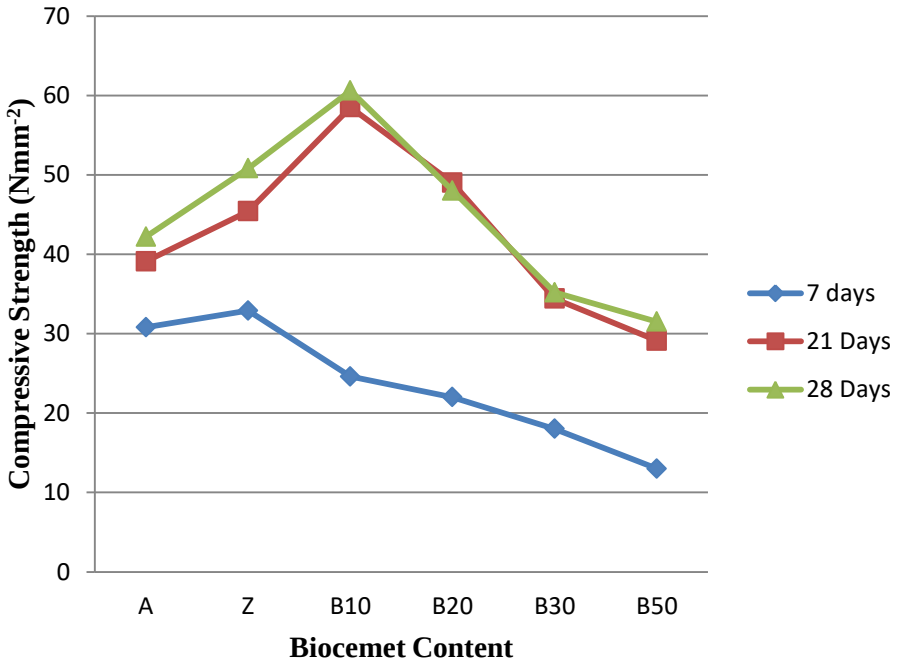

Figure 2: Graph showing the Compressive Strength of mortar prisms

	Collapse Load (kN)							Compressive Strength (N/mm^{-2})
	7 days cured							
	1	2	3	4	5	6	Average	
A	48.2	49.1	48.6	52.7	49.5	47.7	49.3	30.8
Z	50.4	55.4	49.1	51.3	56.7	52.7	52.6	32.9
B10	48.6	50.4	44.1	35.1	25.2	32.4	39.3	24.6
B20	37.8	29.3	32.4	37.8	40.1	33.8	35.2	22.0
B30	27.0	37.8	21.6	27.9	30.2	27.9	28.7	18.0
B50	22.5	17.1	23.9	18.5	12.6	30.6	20.9	13.0
	21days cured							
A	66.6	55.4	62.6	51.3	73.4	66.6	62.6	39.1
Z	69.3	66.6	79.2	73.8	75.6	71.6	72.7	45.4
B10	82.8	109.4	86.4	99.5	95.4	88.2	93.6	58.5
B20	77.9	90.9	82.8	80.1	70.7	68.4	78.5	49.0
B30	71.1	47.7	59.4	46.4	53.1	52.2	55.0	34.4
B50	44.1	50.4	45.9	48.6	43.7	46.4	46.5	29.1
	28 days cured							
A	65.3	62.6	75.2	71.1	62.1	69.3	67.6	42.2
Z	67.1	73.4	77.9	89.1	91.8	88.7	81.3	50.8
B10	107.1	89.6	116.6	93.2	90.5	84.6	96.9	60.6
B20	82.4	77.9	76.5	86.4	66.6	70.7	76.7	48.0
B30	73.8	53.1	57.6	54.5	50.9	47.7	56.3	35.2
B50	49.5	59.4	50.9	46.8	43.2	52.7	50.4	31.5

Compressive Constant = $10^3/1600 = 0.625mm^{-2}$

Table 3: Compressive Strengths of Mortar Prisms

CONCLUSION

Based on the findings of this research, it can be concluded that *Bacillus Licheniformis* is a suitable micro-organism for the improvement of the compressive strength of mortar. There is irregular increase in flexural strength of mortar with increase in biocement up to 10% replacement but a steady increase in flexural strength as noticed with 20 to 50% replacement and as the curing days increased from 7 to 28 days curing age. Bacteria cells improved the compressive strength of the mortar by 20% at 28 days curing age. Strength development in biocement was noticed to be slow within 7 days curing age with significant increase for 21 and 28 days.

This study has shown that *Bacillus Licheniformis* is suitable for the precipitation of biocement and the biocement produced is suitable to improve the strength of mortar.

REFERENCES

Cacchio P., Ercole C., Cappuccio G., Lepidi A., (2003). Calcium carbonate precipitation by bacterial strains isolated from a limestone cave and from a loamy soil, Geomicrobiology 20, 85–99.

Castanier, S., Le Metayer-Levrel, G., Perthuisot, J.P., (1999). Ca-carbonates precipitation and limestone genesis – the microbiogeologist point of view. Sed. Geol., 126, 9–23.

Chahal, N., Siddique, R & Rajor, A. (2012). Influence of bacteria on the compressive strength, water absorption and rapid chloride permeability of fly ash concrete. Construction and Building Materials 28(1): 351–356.

DeJong J.T., Mortensen B.M., Martinez B.C., Nelson D.C., (2010). Bio-mediated soil improvement, Ecol. Eng. 36 197–210.

DeGraef B, deWindt W, Dick J, Verstraete W, deBelie N (2005). Cleaning of concrete fouled by lichens with the aid of Thiobacilli. Materials and Structures 38(284): 875–88.

DeMuynck W, deBelie N, Verstraete W (2007). Improvement of concrete durability with the aid of bacteria. Proceedings of the First International Conference on Self-Healing Materials 18–20 April 2007, Noordwijkaan Zee, The Netherlands.

Douglas, S & Beveridge, T. (1998). Mineral formation by bacteria in natural microbial communities. FEMS Microbiology Ecology 26(2): 79–88.

Edvardsen C (1999). Water permeability and autogenous healing of cracks in concrete. ACI Materials Journal 96(4): 448–454.

Elmanama, A. A. and Alhour M. T. (2013). Isolation, Characterization and Application of Calcite Producing Bacteria from Urea Rich Soils. *Journal of Advanced Science and Engineering Research*, *3(4)*, 388-399

Kroll, R. G. (1990). Alkalophile in C, Edwards (Ed), Microbiology of extreme environments. New York, NY: McGraw-Hill.

Le Metayer-Levrel, G., Castanier, S., Orial, G., Loubiere, J.F.et al., (1999). Applications of bacterial carbonatogenesis to the protection and regeneration of limestones in buildings and historic patrimony. Sed. Geol., 126, 25–34.

Li VC, Yang E (2007). Self-healing in concrete materials. In S. van der Zwaag (ed.) *Self-healing materials—Analternative approach to 20 centuries of materials science*. Springer, The Netherlands; pp: 161–194.

Lian L., Hu Q., Chen J., Ji J., Teng H.H., (2006). Carbonate biomineralization induced by soil bacterium Bacillus megaterium, Geochim.Cosmochim.Acta70 5522–5535.

Lopez-Garcia P., Kazmierczak J., Benzerara K., Kempe S., Guyot F. (2005). Moreira, Bacterial diversity and carbonate precipitation in the giant microbialites from the highly alkaline Lake Van, Turkey, Extremophiles 9 263–274.

Neville AM (2002). Autogenous healing—A concrete miracle *Concrete Int* 24(11):76–82. organic acids: expanding the markets. *Trends in Biotechnology*, *26*(2), 100-108.

Okafor F.O and Ewa.D. (2012). Predicting the Compressive Strength of Obudu Erath Blocks Stabilised with Cement Kiln Dust.

Oyekan, G.L and Kamiyo O.M. (2008). A study on the Engineering Properties ofsandcrete blocks produced JETR 3(3), 88-98.

Reinhardt H.W, Jooss M (2003). Permeabilitiy and selfhealing of cracked concrete as a function of temperature and crack width. Cement and Concrete Res 33:981–985.

Rivadeneyra M.A., Delgado G., Ramos-Cormenzana A., Delgado R., (1997). Precipitation of carbonates by Deleyahalophila in liquid media: pedologicalimplications in saline soils, AridSoil Res. Rehabil.11 35–49

Rivadeneyra M.A., Delgado G., Ramos-Cormenzana A., Delgado R., (1998). Biomineralisation of carbonates by Halomonaseurihalinain solid and liquid media with different salinities: crystal formation sequence, Res.Microbiol.149 277–287.

Shen Y., Buick R., Canfield D.E., (2001). Isotopic evidence for microbial sulfate reduction in the early Archaean era, Nature 410 77–81.

Stocks-Fischer, S., Galinat, J.K., Bang, S.S., (1999). Microbiological precipitation of $CaCO_3$. Soil Biol. Biochem., 31, 1563–1571.

Whiffin V.S., van Paassen L., Harkes M.P., (2007). Microbial carbonate precipitation as a Soil improvement technique, Geomicrobiology 24 417–423.

Wright D.T., Oren A., (2005). Non-photosynthetic bacteria and the formation of carbonate and evaporate through time, Geomicrobiology 22 27–53.