Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Biocementation of soil by calcite/aragonite precipitation using *Pseudomonas azotoformans* and *Citrobacter freundii* derived enzymes

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Supplementary Table 1: strains identified using BCL card (Gram positive spore forming bacilli)

Sample/test	A1	B1	B2	Y1a	Y2a	
BXYL	-	-	-	-	-	**BXYL** (betaxylosidase),
LysA	-	-	-	-	-	**LysA** (L- lysine arylamidase),
AspA	-	-	-	+	-	**AspA** (L aspartate arylamidase),
LeuA	+	+	+	+	+	**LeuA** (leucine arylamidase),
PheA	-	+	-	-	-	**PheA** (phenylalanine arylamidase),
ProA	-	-	-	+	-	**ProA** (L-proline arylamidase),
BGAL	-	-	-	-	-	**BGAL** (beta galactosidase),
PyrA	+	+	+	+	+	**PyrA** (L-pyrrolydonyl arylamidase),
AGAL	-	-	-	-	-	**AGAL** (alpha galactosidase),
AlaA	-	-	-	+	-	**AlaA** (alanine arylamidase),
TyrA	-	-	-	-	-	**TyrA** (tyrosine arylamidase),
BNAG	+	+	+	+	+	**BNAG** (beta-N-acetyl glucosamindase),
APPA	+	+	+	+	+	**APPA** (Ala-Phe-Pro arylamidase),
CDEX	-	-	+	-	-	**CDEX** (cyclodextrin),
Dgal	+	-	-	-	-	**dGAL** (D-galactose),
GLYG	+	-	-	-	-	**GLYG** (Glcogen),
INO	-	-	-	-	-	**INO** (myo-inositol),
MdG	-	-	-	-	-	**MdG** (Methyl-A-D-Glucopyranoside acidification),
ELLM	-	-	-	-	-	**ELLM** (Ellman),
MdX	-	-	-	-	-	**MdX** (Methyl-D-Xyloside),
AMAN	-	-	-	-	-	**AMAN** (Alpha-Mannosidase),
MTE	+	+	+	-	+	**MTE** (Maltotriose),
GlyA	-	-	-	+	-	**GlyA (**Glycine Arylamidase),
dMAN	-	-	+	-	-	**dMAN** (D-Mannitol),
dMNE	+	+	+	-	+	**dMNE** (D-mannose),
dMLZ	-	-	+	-	-	**dMLZ** (D-Melezitose),
NAG	+	+	+	-	+	**NAG** (N-Acetyl-D-Glucosamine),
PLE	-	-	-	-	-	**PLE** (Palatinose),
IRHA	-	-	-	-	-	**IRHA** (L-Rhamnose),
BGLU	-	-	+	-	-	**BGLU** (Beta-Glucosidase),
BMAN	-	-	-	-	-	**BMAN** (Beta-Mannosidase),
PHC	-	-	-	-	-	**PHC** (Phosphoryl Choline),
PVATE	+	+	+	-	+	**PVATE** (Pyruvate),
AGLU	-	-	-	-	-	**AGLU** (Alpha- Glucosidase),
dTAG	-	-	-	-	-	**dTAG** (D-Tagatose),
dTRE	+	+	+	-	+	**dTRE** (D-Trehalose),
INU	-	-	-	-	-	**INU** (Inulin),
dGLU	+	+	+	-	+	**dGLU** (D-Glucose),
dRIB	+	+	+	-	+	**dRIB** (D-Ribose),
PSCNa	-	-	-	-	-	**PSCNa** (Putrescine assimilation),
NaCl 6.5%	+	+	+	-	+	**NaCl 6.5%** (growth in 6.5% NaCl),
KAN	-	-	+	+	-	**KAN** (Kanamycin Resistance),
OLD	-	-	-	+	-	**OLD** (Oleandomycin Resistance),
ESC	+	+	+	-	+	**ESC** (Esculin hydrolysis),
TTZ	+	+	+	+	-	**TTZ** (Tetrazolium red),
POLYB-R	+	+	+	+	+	**POLYB-R** (Polymixin B resistance)

Supplementary Table 2: strains identified using GN card (Gram negative fermenting and non-fermenting bacilli)

Sample/test	C	S2a	Y1b	Y2b	Y3a	
AAPA	+	+	-	-	-	**APPA** (Ala-Phe-Pro-Arylamidase),
ADO	-	-	-	-	-	**ADO** (Adonitol),
PyrA	+	+	-	+	+	**PyrA** (L-Pyrrolydonyl-**Arylamidase**),
IRAL	-	-	-	-	-	**IARL** (L-Arabitol),
dCEL	-	-	-	-	-	**dCEL** (D-Cellobiose),
BGAL	-	-	-	+	+	**BGAL** (Beta-Galactosidase),
H_2S	-	-	-	+	+	**H_2S** (H_2S Production),
BNAG	+	-	+	-	-	**BNAG** (Beta-N-Acetyl-Glucosaminidase),
AGLTp	+	-	-	-	-	**AGLTp** (Glutamyl Arylamidase pNA),
dGLU	-	-	-	+	+	**dGLU** (D-Glucose),
GGT	-	-	-	-	-	**GGT** (Gamma-Glutamyl-Transferase),
OFF	-	-	-	-	-	**OFF** (Fermentation/Glucose),
BGLU	-	-	-	-	-	**BGLU** (Beta-Glucosidase),
dMAL	-	-	-	+	+	**dMAL** (D-Maltose),
dMAN	-	-	-	+	+	**dMAN** (D-Mannitol),
dMNE	-	-	-	+	+	**dMNE** (D-Mannose)
BXYL	-	-	-	-	-	**BXYL** (Beta-Xylosidase),
BAIap	-	-	-	-	-	**BAIap** (Beta-Alanine arylamidase pNA),
ProA	-	+	+	-	-	**ProA** (L-Proline Arylamidase),
LIP	-	-	-	-	-	**LIP** (Lipase),
PLE	+	-	-	-	-	**PLE** (Palatinose),
TyrA	-	-	-	+	+	**TyrA** (Tyrosine Arylamidase),
URE	+	+	-	-	-	**URE** (Urease),
dSOR	-	-	-	+	+	**dSOR** (D-Sorbitol),
SAC	-	-	-	+	+	**SAC** (Saccharose/sucrose),
dTAG	+	-	-	+	+	**dTAG** (D-Tagatose),
dTRE	-	-	-	+	+	**dTRE** (D-Trehalose),
CIT	-	-	-	+	+	**CIT** (Citrate (sodium)),
MNT	-	-	-	-	-	**MNT** (Malonate),
5KG	-	-	-	+	+	**5KG** (5-Keto-D-Gluconate),
ILTAK	-	-	-	-	-	**ILATk** (L-Lactate alkalinisation),
AGLU	-	-	+	-	-	**AGLU** (Alpha-Glucosidase),
SUCT	+	-	-	+	+	**SUCT** (Succinate alkalinisation),
NAGA	-	-	-	-	-	**NAGA** (Beta-Acetyl-Galactosaminidase),
AGAL	-	-	-	+	+	**AGAL** (Alpha-Galactosidase),
PHOS	+	-	-	-	-	**PHOS** (phosphatase),
GlyA	-	-	-	-	-	**GlyA** (Glycine Arylamidase),
ODC	-	-	-	-	-	**ODC** (Ornithine Decarboxylase),
LDC	-	-	-	-	-	**LDC** (Lysine decarboxylase),
IHISa	-	-	-	-	-	**IHISa** (L-Histidine assimilation),
CMT	-	+	-	+	+	**GMT** (Coumrate),
BGUR	-	-	-	-	-	**BGUR** (Beta-Glucoronidase),
O129R	-	-	+	+	+	**O129R** (O/129 Resistance (comp.vibrio)),
GGAA	-	-	-	-	-	**GGAA** (Glu-Gly-Arg-Arylamidase),
IMLTa	-	-	-	-	-	**IMLTa** (L-Malate assimilation),
ELLM	+	-	-	-	-	**ELLM** (Ellman),
ILATa	-	-	-	+	+	**ILATa** (L-Lactate assimilation).

Supplementary Table 3: strains identified using GP (Gram positive cocci and non-spore forming bacilli

Sample/test	S2b	Po_4	Fe	Y3b	
AMY	-	-	-	-	**AMY** (D-Amygdaline),
PIPLC	-	-	-	-	**PIPLC** (Phosphatidylinositol phospholipase C),
dXYL	+	-	-	-	**dXYL** (D-Xylose),
ADH1	+	-	-	-	**ADH1** (Arginine Dihydrolase 1),
BGAL	+	-	-	+	**BGAL** (Beta-Galactosidase),
AGLU	-	-	-	-	**AGLU** (Alpha-Glucosidase),
APPA	-	-	-	-	**APPA** (Ala-Phe-Pro-Arylamidase),
CDEX	-	-	-	-	**CDEX** (Cyclodextrine),
AspA	-	-	-	-	**AspA** (L-Aspartate Arylamidase),
BGAR	-	-	-	-	**BGAR** (Beta Galactopyranosidase),
AMAN	-	-	-	-	**AMAN** (Alpha-Mannosidase),
PHOS	-	-	-	-	**PHOS** (Phosphatase),
LeuA	+	-	-	-	**LeuA** (Leucine Arylamidase),
ProA	-	-	-	-	**ProA** (L-Proline Arylamidase),
BGURr	-	-	-	-	**BGURr** (Beta glucuronidase),
AGAL	-	-	-	+	**AGAL** (Alpha Galactosidase),
PyrA	+	-	-	-	**PyrA** (L-Pyrrolidonyl-Arylamidase),
BGUR	-	-	-	-	**BGUR** (Beta-glucuronidase),
AlaA	+	-	-	-	**AlaA** (Alanine Arylamidase),
TyrA	-	-	-	-	**TyrA** (Tyrosine Arylamidase),
dSOR	-	-	-	-	**dSOR** (D-Sorbitol),
URE	-	-	-	-	**URE (**Urease),
POLYB	+	-	-	-	**POLYB** (Polymixin B resistance),
dGAL	+	-	-	+	**dGAL** (D-Galactose),
dRIB	+	+	+	-	**dRIB** (D-Ribose),
ILATk	+	-	-	-	**ILATk** (L-Lactate alkalinization),
LAC	+	-	-	-	**LAC** (Lactose),
NAG	+	+	+	-	**NAG** (N-Acetyl-D-glucosamine),
dMAL	+	+	+	+	**dMAL** (D-Maltose),
BACI	+	+	+	-	**BACI** (Bacitracin resistance),
NOVO	+	-	-	-	**NOVO** (Novobiocin resistance),
NC6.5	+	-	-	-	**NC6.5** (Growth in 6.5% NaCl),
dMAN	+	-	-	+	**dMAN** (D-Mannitol),
dMNE	+	-	-	-	**dMNE** (D-Mannose),
MBdG	+	-	-	+	**MBdG** (Methyl-B-D-g-Glucopyranoside),
PUL	-	-	-	-	**PUL** (Pullulan),
dRAF	-	+	+	+	**dRAF** (D-Raffinose),
O129R	+	+	+	+	**O129R** (O/129 Resistance (comp vibrio)),
SAL	+	+	+	-	**SAL** (Salicin),
SAC	-	+	+	+	**SAC** (Saccharose/sucrose),
dTRE	+	+	+	+	**dTRE** (D-Trehalose),
ADH2s	-	-	-	-	**ADH2s** (Arginine Dihydrolase 2),
OPTO	+	-	-	+	**OPTO** (Optochin resistance).

Supplementary Table 4. The standard XRD data of $CaCO_3$ with calcite (JCPDS no. 47-1743), and aragonite (JCPDS no. 41-1475).

Reference calcite

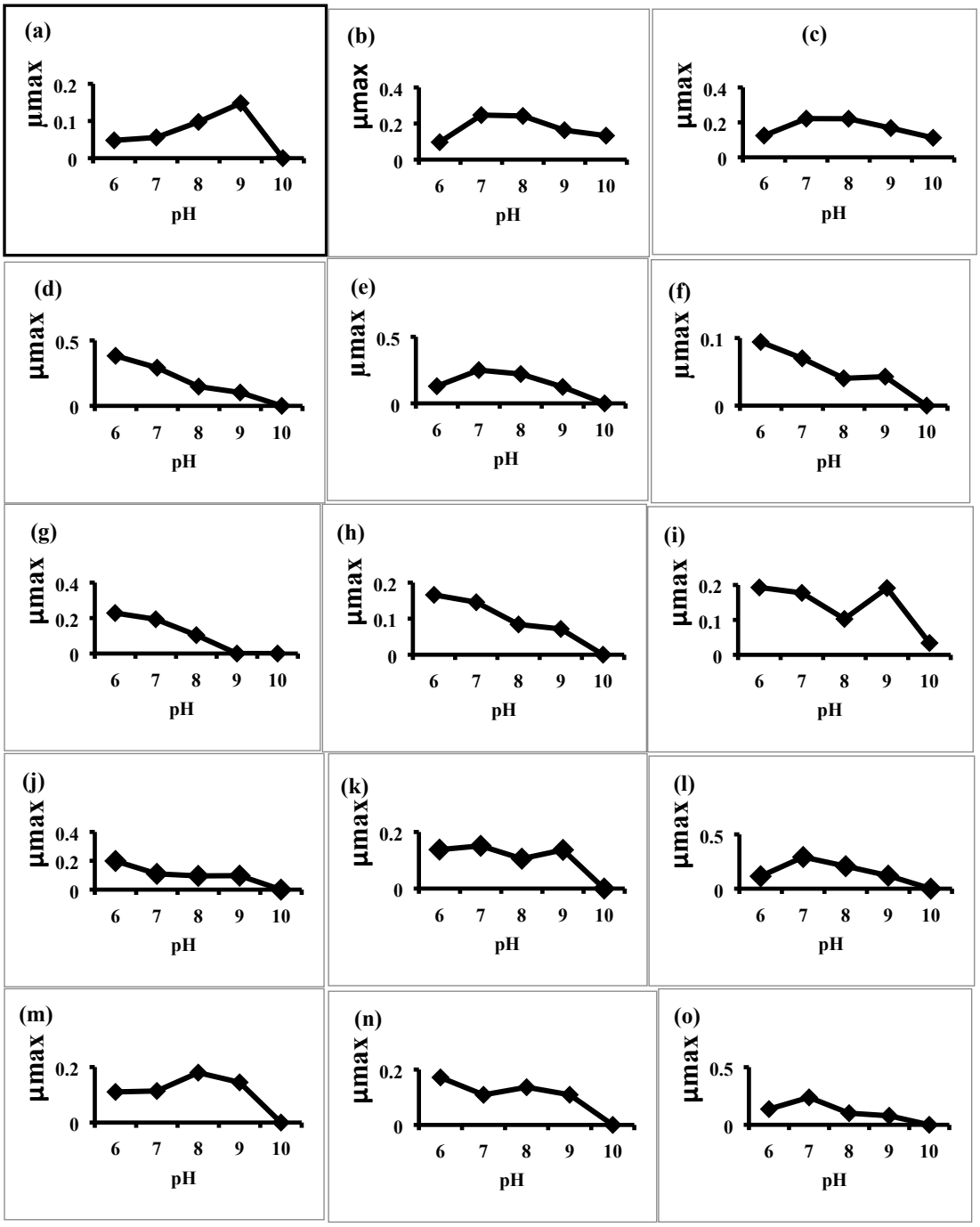
No.	h	k	l	d [A]	2Theta[deg]	I [%]
1	0	1	2	3.85480	23.054	8.9
2	1	0	4	3.03550	29.401	100.0
3	0	0	6	2.84350	31.435	2.2
4	1	1	0	2.49480	35.969	14.7
5	1	1	-3	2.28460	39.409	20.2
6	2	0	2	2.09440	43.159	16.1
7	0	2	4	1.92740	47.114	7.0
8	0	1	8	1.91240	47.506	21.7
9	1	1	-6	1.87530	48.505	23.2
10	1	2	-1	1.62580	56.562	4.2
11	1	2	2	1.60410	57.398	11.8
12	1	0	10	1.58690	58.079	1.2
13	1	2	-4	1.52520	60.669	6.6
14	2	0	8	1.51780	60.996	3.0
15	1	1	-9	1.50940	61.372	3.6
16	2	1	-5	1.47320	63.051	2.7
17	3	0	0	1.44040	64.658	8.4
18	0	0	12	1.42180	65.610	4.8
19	1	2	-7	1.35680	69.185	1.9
20	0	2	10	1.33900	70.238	3.1
21	2	1	-8	1.29670	72.889	3.7
22	0	3	6	1.28490	73.668	0.8
23	2	2	0	1.24740	76.271	1.5
24	1	1	-12	1.23520	77.162	2.8
25	1	3	-2	1.18680	80.941	0.8
26	1	2	-10	1.17980	81.522	3.7
27	0	1	14	1.17290	82.105	0.5
28	1	3	4	1.15380	83.767	6.8
29	2	2	-6	1.14230	84.806	3.5
30	2	1	-11	1.12470	86.455	0.9
31	2	0	14	1.06140	93.061	1.4
32	4	0	4	1.04720	94.713	4.9
33	1	3	-8	1.04480	94.999	5.3
34	1	0	16	1.03520	96.165	2.5
35	1	1	-15	1.03490	96.202	0.8
36	1	2	-13	1.02300	97.699	0.6
37	3	0	12	1.01180	99.161	5.4
38	2	3	-1	0.98970	102.213	0.7
39	3	2	-2	0.98470	102.938	2.7
40	3	1	-10	0.98070	103.526	0.6
41	2	1	-14	0.97670	104.124	2.4
42	2	3	-4	0.96560	105.830	1.8
43	0	4	8	0.96370	106.130	4.4
44	0	2	16	0.95620	107.334	1.0
45	3	2	-5	0.95200	108.024	0.6
46	4	1	0	0.94290	109.561	4.2
47	2	2	-12	0.93770	110.466	1.9
48	2	3	-7	0.91830	114.034	0.6
49	3	2	-8	0.89900	117.927	1.9
50	4	1	-6	0.89500	118.784	1.8
51	1	2	-16	0.89290	119.241	2.2
52	1	1	-18	0.88600	120.781	2.8
53	5	0	2	0.85980	127.250	0.6
54	2	3	-10	0.85710	127.983	3.2
55	2	1	-17	0.85510	128.535	0.6
56	1	3	-14	0.85450	128.702	0.8
57	0	5	4	0.84700	130.858	2.5
58	0	1	20	0.83690	133.975	3.1
59	3	2	-11	0.83530	134.495	0.8
60	3	3	0	0.83160	135.726	1.6

Reference aragonite

No.	h	k	l	d [A]	2Theta[deg]	I [%]
1	0	0	4	4.22600	21.005	25.0
2	1	1	0	3.57300	24.900	60.0
3	1	1	2	3.29400	27.048	100.0
4	1	1	4	2.73000	32.778	90.0
5	2	1	1	2.31800	38.818	5.0
6	2	0	5	2.28200	39.456	2.0
7	1	1	6	2.21200	40.759	5.0
8	2	1	3	2.16100	41.765	2.0
9	0	0	8	2.11300	42.760	20.0
10	3	0	0	2.06300	43.849	60.0
11	3	0	4	1.85400	49.099	30.0
12	1	1	8	1.82000	50.079	70.0
13	2	2	0	1.78800	51.039	5.0
14	2	2	2	1.75000	52.230	2.0
15	2	2	4	1.64600	55.807	30.0
16	4	0	1	1.54400	59.854	5.0
17	2	2	6	1.51000	61.345	1.0
18	3	0	8	1.47700	62.870	10.0
19	0	0	12	1.41300	66.070	2.0
20	2	2	8	1.36600	68.653	10.0
21	4	1	0	1.35100	69.524	1.0
22	4	1	2	1.33500	70.480	1.0
23	1	1	12	1.31100	71.969	20.0
24	4	1	4	1.28600	73.595	20.0
25	3	3	0	1.19000	80.678	5.0
26	3	0	12	1.16300	82.957	5.0
27	3	3	4	1.14600	84.469	10.0
28	4	1	8	1.13800	85.202	10.0
29	2	2	12	1.10900	87.989	10.0
30	0	0	16	1.05700	93.565	5.0
31	6	0	0	1.03200	96.562	1.0
32	1	1	16	1.01400	98.869	5.0
33	6	0	4	1.00200	100.486	1.0
34	5	2	0	0.99090	102.042	1.0
35	4	1	12	0.97490	104.396	5.0
36	5	2	4	0.96490	105.940	2.0
37	3	0	16	0.94100	109.889	10.0
38	3	3	12	0.91010	115.642	2.0
39	2	2	16	0.91010	115.642	2.0

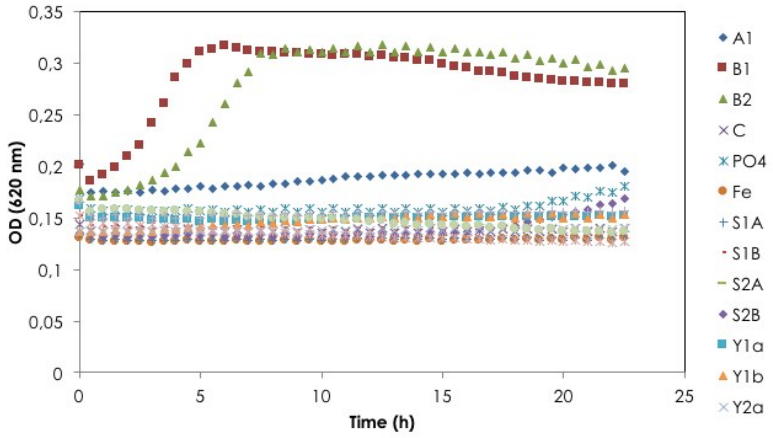
Supplementary Fig. 1.

Supplementary Fig. 1A: Optimum pH curve for (a) A1 strain, (b) B1 strain, (c) B2 strain, (d) C strain, (e) PO_4 strain, (f) S1a strain, (g) S1b strain, (h) S2a strain, (i) S2b strain, (j) Y1a strain, (k) Y1b strain, (l)Y2a strain, (m) Y2b strain, (n) Y3a strain, (o) Y3b strain.

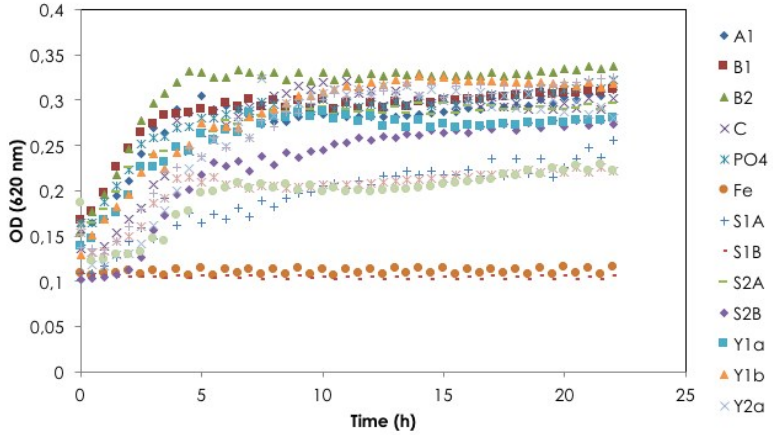


Supplementary Fig. 1B. Growth curves of the different isolates at different pH values.

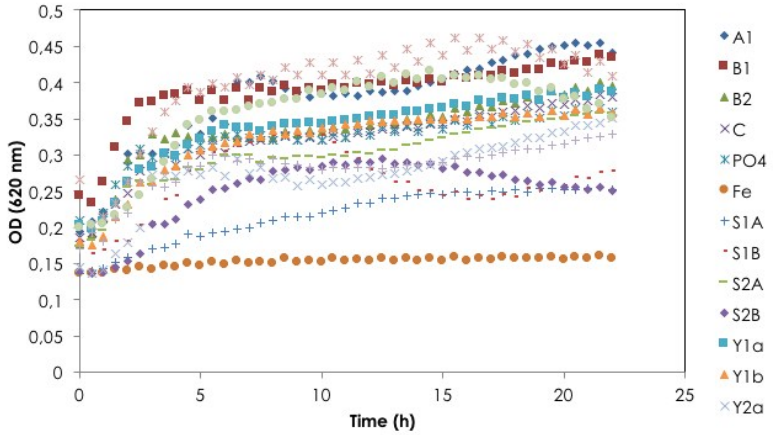
pH 10



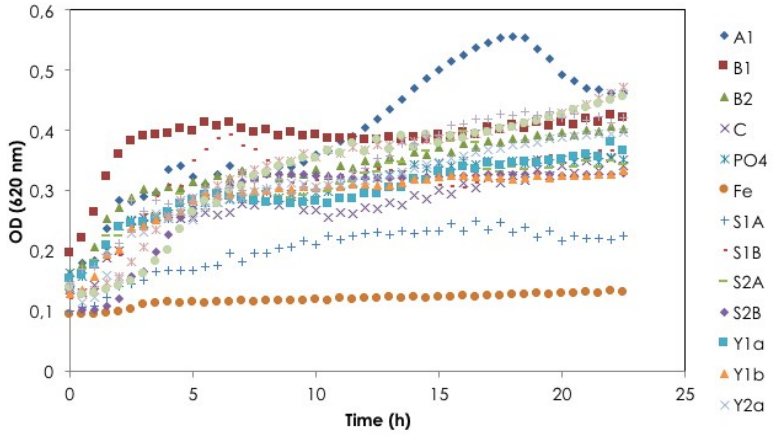
pH 9



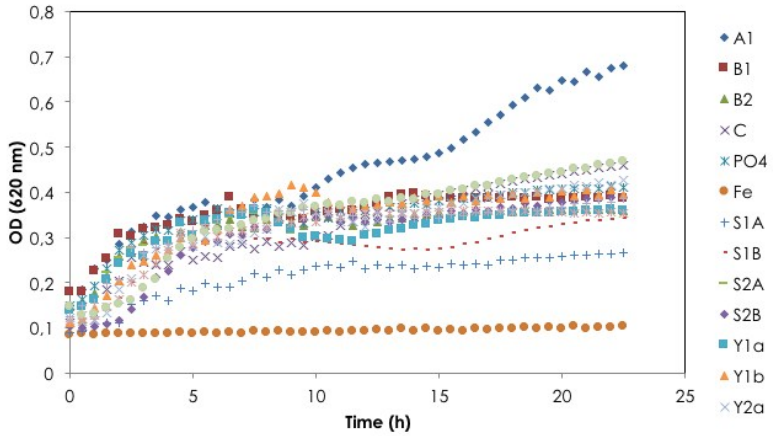
pH 8



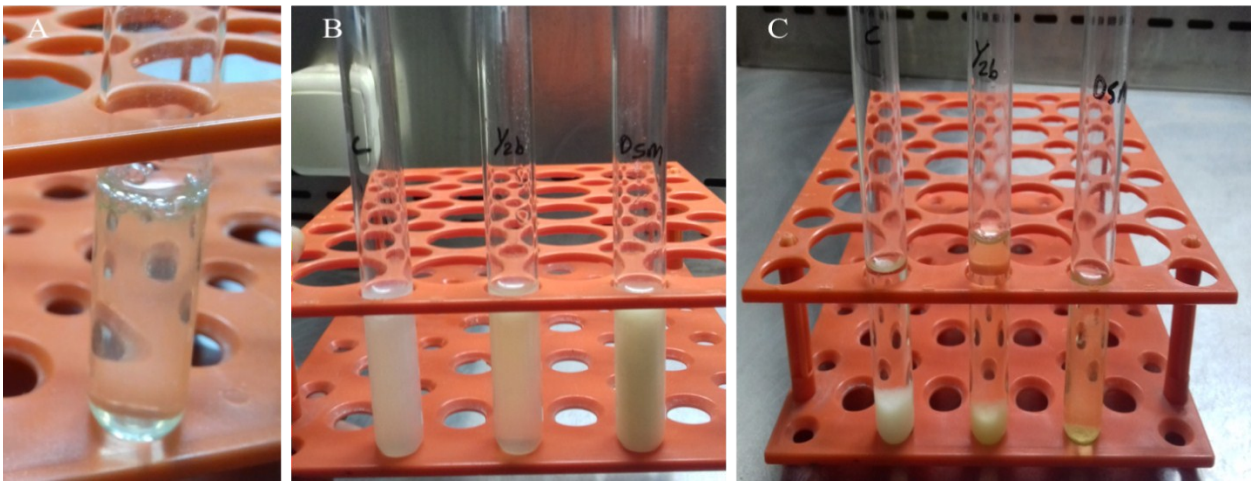
pH 7


OD (620 nm)
Time (h)
A1
B1
B2
C
PO4
Fe
S1A
S1B
S2A
S2B
Y1a
Y1b
Y2a

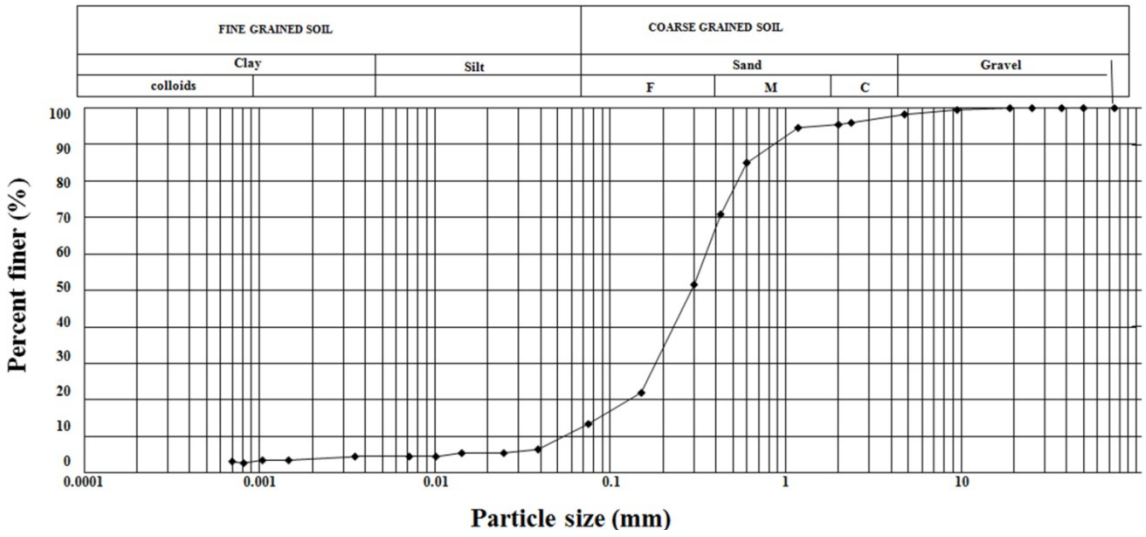
pH 6


OD (620 nm)
Time (h)
A1
B1
B2
C
PO4
Fe
S1A
S1B
S2A
S2B
Y1a
Y1b
Y2a

Supplementary Fig. 2. (A) Negative control, (B) calcium carbonate crystal formation after immediate reaction of samples' supernatant and cementation solution, (C) Calcite precipitate after one week. Tubes order: Left (Isolate C), Middle (Isolate Y2b), Right (Reference strain DSM 33).



Supplementary Fig. 3. Particle size distribution curve



Supplementary Fig. 4. The standard XRD data of $CaCO_3$ with calcite (JCPDS no. 47-1743), vaterite (JCPDS no. 33-0268) and aragonite (JCPDS no. 41-1475)